Evolution of *Nassauvia* Comm. ex Juss. (Asteraceae; Nassauvieae): new insights from old data

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ABSTRACT

The present work collated and reanalyzed DNA sequences for species of *Nassauvia* Comm. ex Juss. (including erstwhile *Triptilion* Ruiz & Pav.) (Asteraceae, Nassauvieae) reported in several previously published phylogenetic analyses. These sequences included: **(i)** the nuclear ribosomal DNA internal transcribed spacer region (ITS) and **(ii)** 5' external transcribed spacer (ETS), and **(iii)** the plastome (cpDNA) *rpl32-trnL*(UAG) intergenic spacer (*rpl32-trnL*), **(iv)** *trnL*(UAA) intron plus *trnL*(UAA)-*trnF*(GAA) intergenic spacer (*trnL-trnF*), and **(v)** *ndhF*. Several low-quality and substantially erroneous sequences were found and discarded, and noisy flanking regions characterizing most sequences were trimmed. A few sequences are aberrant taxonomically, and some contained spurious but analytically innocuous deletions. Following filtering/cleaning of bad data, the consequent analysis **(1)** reconciled discordant topologies published previously; **(2)** confirms earlier findings that erstwhile *Triptilion* is nested within *Nassauvia*, hence is included here within *Nassauvia*; **(3)** demonstrates that species formerly classified in *Triptilion* are polyphyletic within *Nassauvia*, hence are classified here in two separate *Nassauvia* sections; (**4)** demonstrates that phylogenetic relations suggested by nuclear and cpDNA sequences for species formerly classified in *Triptilion* may be incongruent; **(5)** demonstrates and analyzes possible nuclear/cpDNA tree incongruence at the intersectional level; **(6)** confirms polyphyly of sequences with relation to the sectional classification; **(7)** reports sequence polymorphism and polyphyly at the infraspecific levels; **(8)** reports possible intersectional hybridization; **(9)** otherwise analyzes the historical cause of the infraspecifically polyphyletic sequences in general; and **(10)** reevaluates *Nassauvia* taxonomy in light of the present results. But besides merely deferring to *causas proximas* of gene tree incongruencies with each other and with morphology-based taxonomy (viz. hybridization and lineage sorting), the present work emphasizes the *causa ultima*, viz. the ontological distinction between genes and organisms. In particular, while genes and organisms are physically hierarchically linked and mutually interdependent, they otherwise can "drift" evolutionarily as long as the conditions for their quasi-independent existence and capacity for systemic reproduction are maintained. In this way, the genome lineage may be highly polyphyletic, while the morphological/behavioral ontogenetic phenotype of the organismal lineage remains monophyletic. Thus, *some*, apparent incongruencies may be red herrings.
Key words: *Nassauvia*, *Triptilion*, *Calopappus*, Asteraceae, Mutisioideae, Nassauvieae, gene tree incongruence, species phylogeny, epigenesis.

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Introduction

Since the early 19th Century, based on morphology, the genera *Calopappus* Meyen, *Nassauvia* Comm. ex Juss, and *Triptilion* Ruiz & Pav.[1] (Asteraceae, Nassauvieae) have been considered intimately

[1] *Calopappus* is monotypic, distributed from ca. 30-35S at 2300-3200m (viz. montane to subalpine; Rodriguez et al., 2018). Its single species, *C. acerosus* Meyen, is a (sub)shrub, *lacking basal rosette leaves*, with *short-shoot* stems bearing awl-like sclerophyllous leaves with *parallel venation*, with a *monocephalous* inflorescence bearing capitula

interrelated (Crisci, 1974, 1980, 1982; Cabrera, 1982; Katinas et al., 1992 [hereafter Katinas1992], 2008a [hereafter Katinas2008a]; Freire et al., 1993 [hereafter Freire1993]). This close interrelationship has been confirmed by multiple molecular phylogenetic analyses (Kim et al., 2002; Katinas2008a; Simpson et al., 2009 [hereafter Simpson2009]; Maraner et al., 2012 [hereafter Maraner2012]; Vidal, 2012 [hereafter Vidal2012]; Jara-Arancio et al., 2017a ["2018;" hereafter Jara2017a]; Lavandero et al., 2024 [hereafter Lavandero2024]). The molecular analyses have confirmed earlier conclusions that *Calopappus* is sister to a clade comprising the other two genera (Crisci, 1982; Katinas1992; contra Freire1993), but they have refuted the notion that *Nassauvia* and *Triptilion* are sister taxa (Crisci, 1974; Katinas1992, cf. Katinas2008a; Freire1993). In particular, several analyses have demonstrated that species formerly classified in *Triptilion* are phylogenetically nested among *Nassauvia* species, hence that the latter is not monophyletic. Moreover, most analyses have shown the erstwhile *Triptilion* species to be polyphyletic, with *N. achillea* (DC) Hershk. (≡ *T. achilleae* DC) originating separately from the remaining six species. Hereafter, I apply the taxonomy of Hershkovitz (2025a), which classifies *N. achillea* in *N.* sect. *Achilleae* Hershk. and the remaining erstwhile *Triptilion* species in *N.* sect. *Triptilion* (Ruiz & Pav.) Hershk. In addition, *N.* subg. *Strongyloma* (DC) Cabrera (cf. Nicola et al., 2019 [hereafter Nicola2019]); is classified as *N.* sect. *Strongyloma* (DC) Hershk., while the remaining *Nassauvia* species are classified in the sections established by Cabrera (1982).

While multiple previous analyses demonstrated that classical *Triptilion* and *Nassauvia* species are phylogenetically intermingled, evidence for precise species interrelationships from different studies is contradictory and/or inconclusive. This owes to various analytical problems, including species sampling, analytical method, alignment/sequence accuracy issues, possible sample misidentifications and/or species misdiagnosis, as well as biological phenomena, including deep-level genome tree incongruence and contemporary interspecific gene flow. Besides the *Triptilion* question, the various morphological and molecular analyses also have disagreed regarding relations among both species and the major clades of *Nassauvia*. At the same time, these analyses have cast doubt on the monophyly of the subgeneric taxa established by Cabrera (1982).

Thus, the purpose of the present work is to collate and reanalyze the DNA sequence data from the various studies. This analysis confirms with greater accuracy and certainty earlier results demonstrating that: **(1)** both nuclear and plastome sequences from classical *Triptilion* species originated twice within the

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with *multiseriate* bracts, and with narrowly *non-plicate* paleaceous pappus scales (Crisci, 1982; Katinas et al., 2008b [hereafter Katinas2008b]). Erstwhile *Triptilion* (here classified in *Nassauvia*) included seven species (12 according to Maraner2012), five endemic to lowland to montane Chile, and two extending also into Argentina (Rodriguez et al., 2018). The species are annual or perennial *rosettiform* herbs, (mostly) with aerial stems with *elongated internodes* (heteroblasts) bearing somewhat sclerophyllous and ± *pinnately-veined*/*lobed* leaves/bracts, inflorescences that are variously *pseudo-racemose/corymbose to pseudocephalic*, capitula with *2-3-seriate* bracts, and *plicate paleaceous* pappus scales/bristles (Katinas1992; Katinas2008b). Classical *Nassauvia* includes ca. 40 species (POWO/WCVP [https://powo.science.kew.org/about-wcvp; acc. 5 Jun 2025] cf. Cabrera, 1982; Katinas2008b) distributed from the altiplano of Bolivia, southward along the Andes at mostly (sub)alpine elevations, to southernmost Patagonia, where some species extend eastward into the lowland pampa, and also the Falkland Island (Islas Malvinas; Katinas2008b; Upson et al., 2013). The genus includes perennial herbs, subshrubs, and shrubs, *lacking basal rosette leaves*, with usually *short-shoot* (brachyblast) aerial stems bearing sclerophyllous leaves usually with *parallel venation*, with inflorescences that are variously monocephalous to *pseudo-racemose/corymbose to pseudocephalic*, capitula with *2-seriate* bracts, and variously entire to ciliate and then filiform to plumose to *non-plicate paleaceous* pappus scales/bristles (Freire1993; Katinas2008a). Cabrera (1982) divided *Nassauvia* into two subgenera, *N.* subg. *Strongyloma* (DC) Cabrera (here *N.* sect. *Strongyloma*), five [sub]shrubby and mostly southern Patagonian spp.; Nicola2019) and *N.* subg. *Nassauvia*. He divided the latter into four sections, three polytypic [*N.* sections *Nassauvia*, *Mastigophorus* (Cass.) DC, and *Panargyrum* (Leg.) Weddell], and one monotypic [*N.* sect. *Caloptilium* (Lag.) Bentham & Hook.f.] All of Cabrera's (1982) taxa correspond to originally generic names.

corresponding *Nassauvia* gene trees; (**2)** *N.* subg. *Nassauvia* sensu Cabrera (1982) is not monophyletic and that, at least from the standpoint of gene trees, none of its four polytypic sections are strictly monophyletic; (**3)** considerable incongruence between nuclear and plastome trees at all phylogenetic levels implicates *continuous* processes of hybridization and/or lineage sorting that have been *ongoing* since the origin of this clade; **(4)** theoretical and technical inadequacies/errors in the most recent heavily taxpayer-funded molecular phylogenetic research that, in some ways, hindered more than helped understand the evolution of *Nassauvia*. Aspects of the evolution of *Nassauvia* are reconsidered in light of the present results and broader evolutionary theory.

Materials and Methods

This work undertook phylogenetic analyses of available sequences (Table 1) of the nuclear rDNA 5' external transcribed spacer (ETS), nuclear rDNA internal transcribed spacer region (ITS, including ITS1, ITS2, and the 5.8S large subunit rDNA), plastome (cpDNA) *rpl32-trnL*(UAG) intergenic spacer (*rpl32-trnL*), *trnL*(UAA) intron plus *trnL*(UAA)-*trnF*(GAA) intergenic spacer (*trnL-trnF*), and partial *ndhF*. Henceforth, I use the term "cpDNA-n" to refer to the (mostly) "noncoding" *rpl32-trnL*/*trnL-trnF* data, because, compared to the protein-coding *ndhF* data, species sampling is much better, the data are much more phylogenetically informative, and also because most of these sequences were derived from the same individual as the ITS sequences. I refer separately to "*ndhF*," because these data are available for only six *Nassauvia* (including erstwhile *Triptilion*) species, they are much less informative, and they derive from individuals different from those for the ITS/cpDNA-n sequences.

For various reasons elaborated later and in Table 1, not all available sequences were used in the various individual locus analyses. Among the sequences analyzed in individual locus analyses, not all were included in the various combined data analyses. This is mainly because sequences for all loci were not available for the same sampled individuals, and, in some cases, because of incongruence between nuclear and cpDNA-n trees. The major conclusions of this work were deduced by cross-validating results across all individual locus and combined data analyses.

The separate analyses undertaken are as follows:

1. Separate realignments and analyses of the 58 distinct ITS and as many as 42 distinct *rpl32-trnL* sequences reported by Nicola et al. (2014), Nicola 2019).[2] for the five species *N.* sect. *Strongyloma*. This was in order to confirm the reported results and select exemplar ITS sequences from each of the two ITS clades reported by Nicola2019 for the broader ITS analysis.

2. Separate realignments and analyses of the ETS, ITS, *trnL-trnF*, *rpl32-trnL*, and *ndhF* DNA sequences for *Nassauvia* (including erstwhile *Triptilion*) and *Calopappus*, with each data set including sequences reported by Maraner2012, Jara2017a, and Nicola2019, plus additional sequences available in GenBank for different individuals of the same and additional species.

3. Separate analyses of condensed ITS, *rpl32-trnL*, and combined cpDNA-n alignments. In addition, I performed separate analyses for ITS1 and ITS2. The data were condensed to eliminate sequences that were identical (whether the same or different nominal species) and sequences of the same nominal species that were monophyletic. The combined cpDNA-n analysis lacked *trnL-trnF* sequences not available for three samples (see below). This was not problematic given the relatively low informative variation of *trnL-trnF*.

[2] Nicola reported 42 cpDNA-n haplotypes that include sequences of this and another locus. I did not determine how much of the total haplotype diversity owes to the latter, but this datum is unimportant in the present context.

4. A final analysis of a further condensed data set combining ITS and cpDNA-n sequences. I also performed separate analyses for ITS1+cpDNA-n and ITS2+cpDNA-n. The ITS and cpDNA-n gene trees were incongruent for one *N. magellanica* individual, but the combined sequences were retained because they still demonstrated polyphyly of the two *N. magellanica* samples, lack of a *trnL-trnF* sequence for the latter notwithstanding. The ETS sequences were excluded from the combined analysis because of both inadequate sampling and poor data quality, and the *ndhF* sequences were excluded because of inadequate sampling (see Results).

Analytical procedures were essentially the same as those described in Hershkovitz (2025b). Automated alignments were undertaken using an online MAFFT[3] server (Katoh, 2002) with default parameters. The alignments were edited manually (considerably) using the BioEdit alignment editor (Hall, 2004). Phylogenetic analyses were undertaken using PAUP version 4.0a169 (Swofford, 2003). The separate ETS, ITS, cpDNA-n, and *ndhF* data sets were analyzed using maximum parsimony (MP) and frequentist maximum likelihood (ML). The MP and ML heuristic search (hs) was undertaken using stepwise taxon addition with 10 trees held at each addition step, unlimited maxtrees, the tree bisection-reconnection (TBR) search algorithm, and otherwise PAUP defaults. The ML substitution models were estimated with ModelTest using the data and an MP tree under the AICc criterion (Posada & Buckley, 2004).

In addition to substitution data, I scored ITS and *rpl32-trnL* for, respectively, three and eleven indel characters. These were added to the alignment using DNA characters as proxies, mostly G and T (or N) and, in two cases, also A. The indels thus were appended also to the combined data analyses. Even though the characters are not base substitutions, I did not exclude them from the ML analyses. This is mainly because I considered that their number was too low to affect substitution parameters and, in any case, the GT substitution rate setting is one by default, with other substitutions greater or less than this.

Bootstrap analyses were undertaken using only variable sites in the alignment. This was because of the exceptionally low % of parsimony informative and variable sites in the cpDNA-n sequences (see Results). This rendered extremely inefficient the already inherently slow ML bootstrap analysis, because sites resampled in each replicate were overwhelmingly invariable. This resulted in artificially low bootstrap resolution for any "reasonable" number of replicates. Analyzing only variable sites resolved this problem. Substitution models for the individual data sets thus were re-estimated using variable sites. However, since the ITS and cpDNA-n models and base frequencies are extremely different, the combined ITS/cpDNA-n ML bootstrap was performed using equal substitution rates and base frequencies, with only the gamma parameter estimated.[4] The ML bootstrap performed 300 replicates with neighbor-joining starting trees, maxtrees = 3, and 300 TBR swaps per replicate.[5] The MP bootstrap thus used the same variable site alignments, performing 1000 replicates, 10 trees held at each stepwise taxon addition step, maxtrees set to 100, and otherwise default PAUP parameters.

In addition to the above, I examined base composition statistics in the ITS and cpDNA-n data, and incongruence between the cpDNA-n and ITS data and also between the ITS2 and ITS2, both by visual comparison of bootstrap results and *heuristically* using the ILD test (see discussion in Hershkovitz, 2025b). The ILD test was performed using the same parameters as the MP bootstrap analyses. To further visually explore incongruence between data sets, I constructed NeighborNet diagrams using the Windows 32-bit version of SplitsTree4 (version 4.14.8; Huson & Bryant, 2006).

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[3] https://www.genome.jp/tools-bin/mafft

[4] Cf. Abadi et al. (2019) regarding the adequacy of less parameterized models in ML analysis.

[5] By monitoring the search status, I established that the ML scores improved little if at all during swaps 200-300.

Results

In the Results and Discussion,[6] I apply the subgeneric taxonomy of Hershkovitz (2025a).

1. Alignments and data sets

Each of the alignments described below corresponds to a data set used in the phylogenetic analyses. All are available as supplemental files as indicated.

a. ITS. Preliminary analysis of the 58 *N.* sect. *Strongyloma* distinct ITS sequences reported by Nicola2019 confirmed that these form two subclades, one of which included nucleotypes representative of all five species and the other four of the five species. Nicola2019 discussed possible explanations for this. For *N.* sect. *Strongyloma*, I arbitrarily selected two Nicola2019 nucleotypes from each subclade plus two additional sequences for the broader *Nassauvia* analysis.

Forty-eight of the remaining 60 available ITS sequences were selected for the analysis, yielding a 655 bp alignment[7] of 54 sequences, mostly 640–645 bp in length. The alignment includes sequences from the monotypic *Calopappus*, 27/40 *Nassauvia* species, and 7/7 erstwhile *Triptilion* species. Of the total alignment length, ca. 260 bp correspond to ITS1, 164 to 5.8S rDNA, and, for all but a few shorter sequences, the 5' 220 bp of the ca. 260 total bp ITS2 length. All available sequences included the 5' end of ITS1, but most terminated in ITS2 ca. 42 bp upstream of the canonical 5' end of the 26S rDNA gene.[8] Longer available sequences showed practically no additional informative variation within this 42 base length. Thus, the alignment was truncated at the 42 bp upstream site. The 54-sequence alignment yielded 182 variable (131 parsimony-informative) sites. Three discrete indel characters were added.

For bootstrap and ML analysis, the data set was condensed to 38 sequences by removing sequences that were either identical (regardless of taxonomy) and arbitrarily selecting a single proxy sequence from among two or more nominally conspecific but *variable* sister sequences. Nominally conspecific sequences that were not monophyletic were not removed.[9] The 38-sequence alignment had 181 variable (106 parsimony-informative) sites, plus the three indel characters.

Base composition among parsimony-informative sites varied more or less taxonomically. Typical of angiosperm ITS (Hershkovitz & Zimmer, 1996), the sequences spanning the alignment are, on average, GC-rich (55%). At parsimony-informative sites, among *Nassauvia* sections, %GC was lowest (43%) in *N. lagascae* (*N.* sect. *Caloptilium*) and highest (55–71%) among *N.* sect. *Triptilion* sequences, while *N. achillea* is 49% GC. Other *Nassauvia* have intermediate values (47–54%), but those of *N.* sect. *Panargyrum* are somewhat higher than those of *N.* sect. *Nassauvia*. Most of the variation in %GC content reflects that in %C.

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[6] Fine, skip the next 28 pages of drivel and go straight to the tree figures. I agree; the Results and Discussion should be limited to 1000 words or the number of words in the names of the authors and their affiliations, whichever is greater.

[7] Supplemental File 1. The 38-sequence alignment can be extracted by deletions.

[8] A few sequences (especially those of Nicola2019) were up to 68 bases shorter.

[9] Additional scrutiny of the data following phylogenetic analysis suggests that some of the infraspecific polyphyly apparent in the ITS trees owes to errors in sequences and sample identifications in the original sequence documents and published analyses thereof (see Results and Discussion).

The ITS substitution model estimated by ModelTest over whole sequences is typical for angiosperm ITS data (Hershkovitz, 2024a), with a very high CT (transition) to GT (transversion) ratio (4.3), followed by the AG transition (1.8), with the other transversion ratios < to << than 1. The estimated gamma shape parameter was 0.37. However, the model selected using only variable sites (for purposes described below) was rather different: the CT and AG transition ratios were high, but equal (3.6), the AC and GT transversion ratio 1, and the AT and CG transversion ratios 0.4. Among-site rate variation was not significant, hence gamma was not selected. Average %GC was 43% (viz. AT- rather than GC-rich), and average %T was extremely high (61%). Considering only parsimony-informative sites yielded another set of substitution ratios: CT (9.3), AG (3.5), and all transversion ratios 1. Base frequencies were "equal"[10] and, again, no gamma.

The question emerges as to which model is correct. This question is difficult to answer. In common wham-bam-thank-you-ma'am molecular phylogenetic analytical practice, researchers usually apply ModelTest perfunctorily to whole alignments only. Because that is what everybody *else* does. Differences described above thus never are discovered, and parameter estimates never are questioned. Yet, quite evidently, parameters estimated over whole alignments are different from those estimated at the relevant sites, viz. those that actually *evolve*. Thus, "*…the complex mutational processes producing real sequence diversity are never fully captured by nucleotide substitution models*" (Schwartz & Mueller, 2010: 16). Ultimately, substitution models are not so much estimated as they are arbitrarily *imposed* "*…for statistical tractability rather than being necessarily based on understanding of the underlying evolutionary processes*" (Bromham et al., 2018: 3).

b. ETS. Jara2017a reported ETS sequences for 22 species of *Nassauvia* (including erstwhile *Triptilion*) and *Calopappus*, viz. many fewer than, e.g., for the 34 species they reported or downloaded for ITS. Two additional ETS sequences were available in GenBank for species sampled by Jara2017a. However, the Jara2017a sequences were problematic, to say the least. Evidently the authors followed a cited protocol (Linder, et al., 2000) to amplify the *complete* rDNA intergenic spacer (IGS) region. This includes both the 5'-and 3'-ETS flanking the rDNA cistron, the former ca. 450 bp in length, in addition to IGS. The IGS is more length-variable, but commonly ca. 3 kb of short repeating motifs interspersed among variable lengths of nonrepeating "random" sequence. The ETS itself evolves similarly to ITS, viz. a multicopy locus whose sequences usually homogenize via concerted rDNA evolution (Hershkovitz et al., 1999; but see Álvarez & Wendel, 2003). Evolution of the IGS itself is "complex," and usually it is not used for phylogenetic analysis.

Jara2017a evidently were able to amplify the IGS region for most but not all of the sampled species. They sequenced *only one* strand of the PCR products using only the 18S-R primer, and then evidently "aligned" and analyzed phylogenetically these *entire* raw (noisy) sequences (including IGS repeats), whose length ranged between ca. 200 to ca. 1400 bp. They did not follow *Step 2* of the *standard* ETS protocol, which is to use the preliminary IGS sequences to develop a new ETS-F primer located at the 5' end of the ETS. This permits amplification and quality sequencing of *all* samples in *both* directions.

I was able to more or less rescue and align ETS sequences for 17 of the 22 IGS region sequences reported by Jara2017a (see Table 1). But because of 3' noise, I trimmed the alignment ca. 30 bp upstream of the canonical 5' end of the 18S gene, yielding a 434 bp alignment with 162 variable (101 parsimony-informative) sites. Its information content notwithstanding, I did not incorporate the ETS data into any combined data analyses. This was mainly because of lack of critical samples (in particular for *N.* sect. *Strongyloma*) and persisting concerns about sequence accuracy. Two high quality ETS sequences

[10] As discussed in Hershkovitz (2025), ModelTest's selection of "equal" base frequencies does not imply stationarity, viz. that base frequencies do not vary among samples. "Equal" base frequencies may be selected simply because the base bias at conserved sites, which are excluded, is discarded.

reported elsewhere were available for *Calopappus* and *N. achillea* (Table 1). These were only trivially different from the corresponding sequences reported by Jara2017, so I did not include them in the analysis.

c. cpDNA-n and *ndhF*

***Rpl32-trnL*.** Preliminary analysis of the Nicola2019 *N.* sect. *Strongyloma rpl32-trnL* sequences confirmed their close similarity, but not their monophyly (see Results). Five distinct sequences from this subgenus, one representing each of the five species, were selected for further analysis. This analysis included 33 of the available 38 sequences representing the remaining taxa. The excluded sequences either were identical to included sequences or unusable (Table 1). Thus, the total alignment included 38 sequences,[11] these representing *Calopappus*, all seven erstwhile *Triptilion* species, and 20/40 remaining *Nassauvia* species plus two others (viz. 22/40) whose reported sequences were identical to those of included species.

As described above for ITS, the alignment was condensed to 31 sequences for further analysis. In this alignment, four nominal species are sampled more than once, because their sequences are not monophyletic. As in Hershkovitz (2025b), the alignment begins within the *rpl32* gene 88 bases upstream of the canonical 5' end of the intergenic spacer and ends 32 bases upstream of the canonical 5' end of the *trnL*(UAG) gene. This alignment of mostly 900-910 bp sequences spanned 989 bases. The 38-sequence alignment included 189 variable (90 parsimony-informative) sites, with eleven parsimony-informative indel characters appended. The 31-sequence alignment included 186 (60 parsimony-informative) sites, plus the eleven parsimony-informative indel characters. The proportion of parsimony-informative sites is much less, because 30 sites supported only the relationship between deleted and included taxa.

Base composition varied less in the *rpl32-trnL* than in the ITS sequences. Despite the high (70%) AT content of whole sequences, parsimony-informative sites were neutral to slightly GC-rich. GC at parsimony-informative sites of the *Nassauvia* sections was 48–52%, but 50-56% in *N.* sect. *Panargyrum*. *N.* sect. *Triptilion* sequences were 48–53%, while *N. achillea* was 44%. Perhaps more significant was variation in the AT balance. The ratio of A to AT content ranged from 34–40%, except for *N.* sect. *Strongyloma*, with 59–61%. Substitution ratio estimates were (1 1.1 0.3 0.3 1.1 1) for whole sequences, (1 1.1 1.2 0.7 0.2 1.2) for variable sites, and (1 0.7 0.3 0.3 0.7 1) for parsimony informative sites. But the variance of all of these estimates, especially the last, must be high, given that there were only 71 total parsimony-informative characters. The gamma shape parameter estimate was 0.47 over all sites, 0.96 over variable sites, and excluded from the parsimony-informative site model.

Examination of the *rpl32-trnL* alignment reveals an example of the inadequacy of standard substitution models nearly universally used in modern molecular/genomic phylogenetics. Beginning at alignment site 321, a peculiar feature appears in all of the *N.* sect. *Nassauvia* sequences: an 8-base motif AAAAAAGG perfectly superimposed over the consensus GCCTTTTT sequence of all other taxa (Fig. 1). It is highly unlikely that the former motif evolved from the latter via eight substitutions. Even if it did, certainly it appears to violate the statistical assumption that substitutions are independent and identically distributed (IID). Thus, I consider that this eight base region is *not* aligned in the sense that the superimposed sites are *homologous*.[12] More likely, the motif originated via deletion/expansion or a

[11] Supplemental File 2.

[12] I *presume* that other studies that analyzed *rpl32-trnL* in these taxa, viz. Vidal2012, Jara2017a, and Nicola2019, superimposed these regions as I have done here. This superimposition emerges from automated alignment programs cited in those works. The authors reported no such alignment artifacts, and they did not make their alignments available for scrutiny.

peculiar recombinatorial event. Yet, as superimposed in the alignment, ModelTest would assume that there are eight IID substitutions, and, as such, would tally these into the substitution matrix itself. Nonetheless, for reasons I explain in the Discussion, I retained this superimposition in both the MP and ML analyses.

TrnL-trnF. A total of 34 *trnL-trnF* sequences were located and aligned for these taxa, one was "garbage," and two included only about half of the total aligned length, hence were not included in the resulting 31-sequence alignment. The alignment included *Calopappus*, all seven erstwhile *Triptilion* species, and 19/40 *Nassauvia* species. Although I conducted preliminary MP analysis of this alignment to detect any "anomalies" relative to the *rpl32-trnL* analysis, the variation was too low to warrant a separate alignment file. Instead, a portion of the sequences were selected for the combined cpDNA-n alignment. However, sequences for samples lacking *rpl32-trnL* sequences still had diagnostic value post-analysis. As in Hershkovitz (2025b), the alignment begins within the *trnL*(UAG) intron 37 bases upstream of the 3' end of the first exon and ends five bases downstream of the canonical 5' end of the *trnF*(GAA) gene. This alignment of mostly ca. 800 bp sequences spanned 813 bases and included 28 parsimony-informative sites, but no informative indels.

Combined cpDNA-n. I combined the *rpl32-trnL* and *trnL-trnF* data sets above into a single 27-sequence cpDNA-n data set that included all taxa for which, with three exceptions, sequences for both loci were available. This combined cpDNA-n alignment included 266 variable (88 parsimony-informative) sites plus the eleven *rpl32-trnL* indels. However, 69% of the variation and 78% of the parsimony-informative variation pertains to the *rpl32-trnL* data. But because this data set was incorporated into the combined ITS/cpDNA-n data set, there is no need to provide it separately.

The exceptions were for samples lacking *trnL-trnF* sequences that nonetheless were critical to overall phylogenetic interpretation and for which the corresponding ITS sequences were available for the combined ITS/cpDNA-n analysis. The samples lacking *trnL-trnF* sequences are for *N. darwinii*, *N. fuegiana*, and one of the *N. magellanica* samples. The first two were included to help reinforce evidence for monophyly of their respective clades, and the last to demonstrate polyphyly of the two *N. magellanica* samples. Otherwise, *rpl32-trnL* sequences manifesting nominal species polyphyly were not included in the combined cpDNA-n analysis, because ITS sequences from the same individual were lacking. Hence their relations in the combined data analysis would be exactly the same as in the analysis of cpDNA alone.

Also, the *trnL-trnF* and *rpl32-trnL* sequences for *N. ulicina* are from different individuals, even though available *rpl32-trnL* sequences for this species are heterogeneous (Nicola2019). But this "Frankenstein" sequence is not problematic, since all three available *N.* sect. *Strongyloma trnL-trnF* sequences are identical, and because *trnL-trnF* otherwise demonstrably is much less variable than *rpl32-trnF*. The inclusion of the *N. ulicina* cpDNA-n sequences thus "anchored" these to the *N. fuegiana rpl32-trnL* sequence for the downstream combined ITS/cpDNA-n analysis. This was done in order to include in the combined data analysis data from at least two nominal species of *N.* sect. *Strongyloma*.

NdhF. Kim et al. (2002) undertook an analysis spanning all Asteraceae using 975 bp from the 3' end of the *ndhF* gene. The analysis included sequences from *Nassauvia digitata*, *N. gaudichaudii*, *N. lagascae*, *N. spinosum*. A *Perezia* sequence proxied for the outgroup. Later, complete *ndhF* sequences were reported for *N. pygmaea* (Panero & Funk, 2008), *N. achillea* (Panero & Crozier, 2016), and *Calopappus acerosus* (Panero et al., 2014). These later studies used the sequences as proxies for intergeneric and not interspecific analyses, and they did not incorporate the Kim et al. (2002) data. For purposes of comparison with the more detailed analyses of the other loci, I aligned and analyzed the 975 bp portion available for *Nassauvia* and *Calopappus* taxa, seven sequences in all.

d. Combined ITS and cpDNA-n. The combined ITS/cpDNA-n alignment[13] included 27 taxa, three of which lacked *trnL-trnF* sequences (see above). In this alignment, the proportion of total/parsimony-informative variation (including indel characters) was 39%/55% for ITS (23%32% for ITS1; 16%/23% for ITS2) and 61%/45% for cpDNA-n (18%/11% for *trnL-trnF*, and 43%/34% for *rpl32-trnL*).

2. Phylogenetic results

a. ITS. Figure 2 shows a sample ITS MP phylogram for the 54-sequence alignment. This phylogram is intended to demonstrate the pattern of sequence divergence rather than support for particular clades. Nonetheless, it shows paraphyly/polyphyly of some nominally infraspecific sequences, polymorphism but monophyly of others, as well as cases of shared nominally *interspecific* sequences. Figure 2 includes representative available sequences for all five *N.* sect. *Strongyloma* species[14] and all but a few of the available sequences, these representing 21 of the remaining ca. 35 spp. classified in the remaining six *Nassauvia* sections. Seven of these 21 were sampled once. Of the 14 species sampled 2(-4)x, three are invariant (6 individuals, 6 nucleotypes) and eleven are polymorphic (26 individuals, 24 nucleotypes). I did not scrutinize the invariant species for indel variation. Of the eleven polymorphic species, sequences of seven appear as nonmonophyletic. However, additional scrutiny suggests that some of this apparent polyphyly owes to sequence artifacts or sample misidentification (see Discussion).

Figure 2 also reproduces the results of Maraner2012, which showed sequences of two species that Cabrera (1982) classified in *N.* sect. *Nassauvia* nested within *N.* sect. *Panargyrum*, and those of another two species that Cabrera (1982) classified in *N.* sect. *Mastigophorus* nested within *N.* sect. *Nassauvia*. I did not include here the sequence Maraner2012 reported for *N. axillaris*, which pertains to *N.* sect. *Strongyloma*, but the Maraner2012 sequence maps to *N.* sect. *Nassauvia* and, in fact, alongside the sequences of the two species classified in *N.* sect. *Mastigophorus* (see above and also Nicola2019). I refer to this "aberrant" sequence in the Discussion.

Figure 3 shows the complete ITS bootstrap consensus results for both MP and ML, with BPs indicated also for ITS1 alone. At most nodes, the ML values are rather higher, which is quite common in molecular phylogenetic analysis for reasons discussed in Hershkovitz (2021a).[15] At least the ML

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[13] Supplemental data file 4.

[14] Nicola2019 surveyed ITS diversity among 107 individuals from 45 collections of five species of *N.* sect. *Strongyloma*. Forty-four of the individuals were polymorphic. A total of 166 sequences were generated, yielding 58 nucleotypes (including indel polymorphisms) from the five species. But only two collections/individuals of *N. maeviae* were sampled, and these shared a nucleotype shared with individuals of all four of the other species. Ignoring this yielded still 58 nucleotypes, but from 164 sequences from 105 individuals of just *four* nominal species. That is a shitload of nucleotypes.

[15] In general, many (often hundreds to thousands of) topologies having equal MP scores have will have unequal ML scores (per their model parameters). This is because MP scores are integers that count the number of substitutions in the tree, whereas ML scores are infinitely more precise per the substitution model. Unlike MP, the ML score does not "count" the substitution per se, but rather the likelihood of it occurring where it occurs in the ML tree. Since the *–ln* likelihood is calculated to a precision of several decimal positions, ML will tend to find only a single ML topology within its precision window. MP bootstrap replicates thus will save a very large number of *different* trees, such that the final proportion of saved trees over all replicates supporting a particular node will tend to be less. However, a given MP BP also may be *higher* than the ML BP owing to fundamental differences in the optimization criterion (e.g., either inadequacy of the MP "model" in the case of long branches, base bias, etc., or, conversely, a highly inaccurate ML substitution model). These possibilities must be examined on a case-by-case basis. The present work reports BP values at 156 nodes where at least the MP or (generally) the ML BP is ≥ 50%. At 146/156

bootstrap of both total ITS and ITS1 indicates support for a sister relation between *N.* sections *Nassauvia* and *Panargyrum* (Node A), which is consistent with the ITS results of Maraner2012, Vidal2012, and Lavandero2024. Also in agreement with Fig. 2, Fig. 3 shows strong support for nesting of two "*N.* sect. *Mastigophorus*" samples among *N.* sect. *Nassauvia* sequences, and of two of the "*N.* sect. *Nassauvia*" sequences among the *N.* sect. *Panargyrum* sequences. Also consistent with Vidal2012 and Jara2017a, Fig. 3 supports monophyly of ITS sequences of *N.* sect. *Triptilion*.

Figure 3 also shows insignificant support for a clade that includes *N.* sections *Achilleae*, *Caloptilium*, and *Strongyloma* (Node B). This is consistent with the ITS trees of Maraner2012 and Lavandero2024 but conflicts with the bootstrap results of Simpson2009 and the (pseudo-)Bayesian tree of Jara2017a. A close relation between *N. lagascae* and *N.* sect. *Strongyloma* also was weakly supported in the ITS analysis of Nicola2019, but this study included no erstwhile *Triptilion* sequences. Resolution of the Vidal2012 tree is inadequate to evaluate this relation. Figure 3 also confirms the two "persistent paralog" ITS subclades of *N.* sect. *Strongyloma* found by Nicola2019. As in Nicola2019, the data do not resolve relations of these subclades relative to other *Nassauvia* species. But Fig. 3 adds to the mix the data from erstwhile *Triptilion* species.

Figure 3 also shows evidence for a sister relation between *N. achillea* and *N. lagascae*. This result is in agreement with the ML ITS tree of Lavandero2024 and the combined ITS/cpDNA-n (pseudo-)Bayesian tree of Jara2017a. Vidal2012 did not find support for this result, but her ITS tree is not resolved sufficiently to rule it out. But while this result emerged in the present ITS analysis, only the ML bootstrap of whole ITS sequences yielded "noteworthy" support. This clade is absent in the bootstrap consensus of ITS1 and ITS2 alone. Comparison of Figures 3 and 4 (see below) explains this. Here, it can be seen that ITS2 alone yields weak support for a clade comprising *T.* section *Achilleae*, *Caloptilium*, and *Triptilion*, viz. excluding *N.* sect. *Strongyloma* (cf. Fig. 3). With *N.* sect. *Strongyloma* "out of the way," the attraction between *N. achilleae* and *N. lagascae* is greater (see also later discussion).

Figure 4 shows the MP/ML bootstrap results for analysis of ITS2 alone. It demonstrates weak support for a Node "A'", which is contrary to Node "A" above. Here, the position of *N.* sect. *Panargyrum* is on the alternate branch of the basal split, which is in agreement with all of the cpDNA-n trees. Other differences from the ITS1 bootstrap can be detected, e.g., in the relation among *N.* sections *Achilleae* and *Triptilion* species. These results suggest a degree of historical incongruence in the evolution of ITS1 and ITS2. This will be evaluated below in the results for the combined ITS/cpDNA-n analysis.

Another incongruency between the ITS1 and ITS2 bootstrap trees involves the relations of the annual species *N. gibbosa*. The ITS1 bootstrap (Fig. 3) strongly supports a clade comprising this species and annuals *N. cordifolia* and *N. capillata*. This clade then is sister to a clade comprising the annual *N. berteroi* and the two perennials *N. benaventii* and *N. spinosa*. The ITS2 bootstrap weakly supports monophyly of the four annual *N.* sect. *Triptilion* species, but both the MP and ML BPs are < 70%. Notably, the relations indicated in the ITS1 bootstrap appear in the ITS bootstrap consensus, but with significantly less support. This, along with the incongruencies described above, indicates that ITS1 and ITS2 phylogenies may be truly incongruent. In fact, the ILD test performed with the 54-sequence data set reported mild but significant ($p = 0.03$) incongruence between these regions (but see below).

Figure 4 also shows at least one and sometimes more than one MP BP > 70% and ML BP > 80% separating all of the nominally conspecific nucleotypes. Thus, the nucleotypes themselves are

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(94%) of these nodes MP BP ≤ ML BP, with only 10 cases of MP BP (usually slightly) ≥ ML BP. In all but one case, I consider these MP and ML BPs as statistically the same.

polyphyletic. In one case, this probably owes to sequence error and specimen misidentification. Otherwise, the meaning of this polyphyly depends on the phylogenetic ontology of phenotypic species, as discussed later.

Examined critically, the ITS bootstrap results, in particular the MP results, do not resolve convincingly the relations between *N.* sections *Achilleae* and *Triptilion* species and remaining *Nassauvia* species. If the MP BPs < 70% are ignored, the well-supported polytypic clades of *Nassauvia*, along with the *N. achillea* and *N. lagascae* branches, all break down into a basal polytomy. In this case, it is not even clear that the erstwhile *Triptilion* nucleotypes are para-/polyphyletic. Erstwhile *Triptilion* polyphyly emerges only considering ML BPs > 70%, which minimally renders erstwhile *Triptilion* paraphyletic with respect to *N. lagascae*. But the exact relations of *N. lagascae* in this case remain unresolved, because the ITS2 and total ITS bootstraps mildly disagree. A similar dilemma involves the relations of *N.* sect. *Panargyrum*. This is resolved only by the ML bootstrap for ITS1 and total ITS, but not by any of the MP bootstraps.

To examine the degree to which the Fig. 3 "Clade B" relations were affected by branch artifacts, I repeated the MP/ML bootstraps using only these 12 sequences plus one "least divergent" proxy sequence from *N.* sections *Nassauvia* and *Panargyrum*. This had no appreciable effect on BPs relative to Fig. 3, although MP/ML BPs for the sister relation between *N. achillea* and *N. lagascae* "evened out" to 70%/74%.

b. ETS. Both the MP and ML bootstrap consensuses (Fig. 5) support two major clades within the study group: **(1)** a clade comprising *N. lagascae* as sister to *N.* sect. *Triptilion*; and **(2)** a clade comprising as sister clades *N.* sections *Panargyrum* and *Nassauvia* (the latter including the "*N.* sect. *Mastigophorus*" sample). The relations of the effective "outgroups" *N. achillea* and *C. acerosus* are unresolved in Fig. 5, so each of these and the two well-supported clades are better interpreted as a 4-tomy. The cistronically linked ITS data suggest that the root should be placed one node up, so that *N. achillea*, *N.* sect. *Triptilion*, and *N. lagascae* form a clade.

Rerooted thusly, the tree moderately supports *N. lagascae* as sister to *N.* sect. *Triptilion*. But it does not matter. This relation emerges from the ETS rerooted or not. Thus, the ETS data *do not* support a sister relation between *N. achillea* and *N. lagascae*, which has some support in the ITS ML bootstrap, but not in ITS1 or ITS2 alone. The other observation is that the support for the sister relation between *N.* sections *Panargyrum* and *Nassauvia* agrees with the ITS and ITS1 bootstraps, but not the ITS2 bootstrap. The latter weakly supports different relations well-supported by the cpDNA-n data and all combinations of ITS and cpDNA-n data (see below).

The ETS trees also mimic the ITS2 trees in showing weak support for a sister relation between the annual species *N. berteroi* and the other annual species rather than, as strongly supported by the ITS1 data alone, the perennial species. However, the ETS data include data for only one of the two perennial species, viz. *N. spinosa* (see also further Results and Discussion).

It is unfortunate that there is not at least one ETS sequence for *N.* sect. *Strongyloma*. This rendered impractical the combination of the ETS data with the ITS and cpDNA-n data. The different data sets yielded differing relations for this clade. This, in turn, affected the relations of the other clades to each other. Especially because of the high information content of the ETS, inclusion of these data without a *N.* sect. *Strongyloma* sequence likely would yield analytical artifacts.

c. cpDNA-n. Figure 6 shows a sample MP phylogram for the 38-sequence *rpl32-trnL* alignment. Again, this phylogram is intended to demonstrate the pattern of sequence divergence and not support for particular clades. Just as in the ITS tree, Fig. 6 shows both polyphyly and monophyly of nominally

conspecific divergent haplotypes, as well as identical haplotypes shared by nominally different species. Also as in the ITS tree, Fig. 6 includes representative sequences for all five species of *N.* sect. *Strongyloma*.[16]

Figure 6 shows all other *rpl32-trnL* data available for 15/35 remaining *Nassauvia* species, and ten of these 15 were sampled only once. Notably, sequences of *all* of the five species sampled 2(-4)X are polymorphic (12 individuals, 11 haplotypes). Four of these five polymorphic species appear as nonmonophyletic in Fig. 6. I did not scrutinize these data for indel polymorphisms. Given that the *rpl-32-trnL* sampling is much less than that for ITS, one might surmise that the true degree of infraspecific haplotype polyphyly is under-represented. At the same time, however, *rpl32-trnL* evolves at perhaps half the "rate" of ITS, and there is no recombinatorial evolution.

Figure 7 shows the results for the MP and ML bootstrap analyses of the condensed 31-sequence alignment. The *rpl32-trnL* trees show important and well-supported differences with respect to the ITS trees. Perhaps the most significant difference is that both MP BPs > 75% and ML BPs ≥ 85% support not only paraphyly of *Nassauvia* with respect to erstwhile *Triptilion*, but also two origins of the erstwhile *Triptilion* haplotypes. Here, the *N. achillea* haplotype not only pertains to the *N.* sect. *Strongyloma* clade, but possibly is nested within it, as suggested by the Fig. 5 phylogram. *N.* sect. *Triptilion* pertains to a strongly supported clade that includes also *N. lagascae* and *N.* sect. *Panargyrum*. Note that the relations of *both* of the last two are strikingly different from those indicated in the ITS trees. In the ITS trees, *N. lagascae* associates with *N. achillea* rather than *N.* sect. *Triptilion*, and *N.* sect. *Panargyrum* associates with *N.* sect. *Nassauvia*. However, as in the ITS tree, the *N. pygmaea* samples ("*N.* sect. *Mastigophorus*") nest within *N.* sect. *Nassauvia*. But, unlike the ITS tree, the sequences from the same individuals that are identical for ITS are highly divergent and polyphyletic for cpDNA-n.

Another possible incongruency between the *rpl32-trnL* and the ITS1 bootstrap trees involves (especially) the relations of the annual species *N. gibbosa*. The ITS1 bootstrap (Fig. 3) strongly supports a clade comprising this species and the annual species *N. capillata* and *N. cordifolia*, with this clade as sister to a clade comprising the annual species *T. berteroi* and the perennial species *N. benaventii* and *N. spinosa*. Meanwhile, ITS2 (Fig. 4) and ETS (Fig. 5) weakly support monophyly of the four annual *N.* sect. *Triptilion* species, with *N. berteroi* as sister to a well-supported clade of the three annual species also well-supported by ITS1. *Rpl32-trnL* and, more so when adding *trnL-trnF*, support a *third* topology, viz. *N. gibbosa* as sister to the *N. berteroi*/ *benaventii*/*spinosa* clade. Interestingly, polymorphism and polyphyly of *N. capillata* and *N. cordifolia* ITS/cpDNA sequences appear in a one phylogenetic tree in Vidal2012. But Vidal2012 did not comment on this result, and the sequences are not available (see Discussion).

However, examination of the ITS (Fig. 2) and *rpl32-trnL* (Fig. 6) phylograms reveals a different sort of incongruence that I discussed in Hershkovitz (2025b): divergence incongruence. The ITS sequences of the perennial species *N. benaventii* and *N. spinosa* are almost identical, whereas all other *N.* sect. *Triptilion* interspecific divergences are rather large. But the *rpl32-trnL* divergence between *N. benaventii* and *N. spinosa* is not only large, it is the *most* divergent among *N.* sect. *Triptilion* species. The MP consensus (not shown) shows the relation of these and *N. berteroi* as unresolved. For practical purposes, the ML and MP bootstrap trees (Fig. 7) are also. Notably, similar results emerge in the combined ITS/cpDNA-n analyses (Fig. 9; see below). The relations of *N. gibbosa* supported by ITS and

[16] Nicola2019 surveyed cpDNA-n diversity at two loci, including *rpl32-trnL*, among 107 individuals from 45 collections of five species of *N.* sect. *Strongyloma*. This yielded 31 haplotypes (including indel polymorphisms). Ignoring *N. maeviae*, this yielded 30 haplotypes among 105 individuals of the remaining four nominal species. However, interspecific sharing of haplotypes was much less than for ITS: only 5/31 haplotypes were shared by more than one species, and none were shared by more than two of them.

ITS1 appear in ITS/cpDNA-n and ITS1/cpDNA-n bootstrap consensuses, though with lower BPs than with ITS alone. But these results conflict with those for the ITS2/cpDNA-n consensus (not shown), in which the relations of *N. gibbosa*/*cordifolia*/*capillata* are resolved only weakly in favor of the ITS2 bootstrap consensus. Also notably, the combined *trnL-trnF*/*rpl32-trnL* data (not shown) shows somewhat stronger support for nesting of *N. achillea* within *N.* sect. *Strongyloma* (Fig. 7).

NdhF. Although the *ndhF* data includes only six *Nassauvia* (including erstwhile *Triptilion*) sequences, it is useful because it is the only data set that includes *N. gaudichaudii*. This is the Type of *N.* sect. *Mastigophorus* (Cabrera, 1982), so its phylogenetic relations determine the eventual taxonomic status of this section. For this species, the single MP and ML tree (Fig. 8) reproduces the results as Kim et al. (2002), viz. *N. gaudichaudii* in a strongly supported clade with the *N. lagascae* and *N. spinosa* sequences. There is not available a sequence for *N.* sect. *Panargyrum*, but the position of *N. gaudichaudii* in this tree is precisely where the other cpDNA-n data place *N.* sect. *Panargyrum*. In fact, morphological analysis (Freire1993) places *all* species of *N.* sect. *Mastigophorus* as sisters to a clade comprising *N.* sect. *Panargyrum* and *N. lagascae*. But, unfortunately, Freire1993 includes *Calopappus* in this clade, while excluding erstwhile *Triptilion*, so we have to be careful about cherry-picking trees. However, the *N. pygmaea* sequence is identical to that of *N. digitata*, thus placing it in a clade with *N.* sect. *Nassauvia*, exactly as in the ITS and *rpl32-trnL* trees. Morphology (Freire1993) positions *N.* sect. *Mastigophorus* species remotely from *N.* sect. *Nassauvia*.

The present data indicate that at least cpDNA-n sequences for Cabrera's (1982) *N.* sect. *Mastigophorus* are polyphyletic. But there are important caveats. One is that the identity of the specimen must be confirmed. This species is one of three that occur on the Falkland Islands, one of which was not yet described in 2002 (Upson et al., 2013). However, the other two species seem to pertain to *N.* sect. *Nassauvia*. But, interestingly, the ITS of two other *N.* sect. *Nassauvieae* species map to *N.* sect. *Panargyrum* (see above), and there are no cpDNA-n or *ndhF* sequences of these species for comparison. The other caveat is that there are not nuclear sequence data for *N. gaudichaudii*.

Meanwhile, in contrast to the "species barcode" trees, the *N. achillea ndhF* sequence appears in Fig. 8 as a highly divergent *sister* to *Nassauvia* and *N.* sect. *Triptilion*. Interestingly, that is the same result as for the available ETS data. However, both data sets lack sequences for *N. sect. Strongyloma*, while the ETS data also lack a sequence for *N. gaudichaudii* and the *ndhF* also for *N.* subg. *Panargyrum*. There is some work to do here.

c. Combined ITS and cpDNA-n. The combined analysis included only 27 sequences for which both ITS and *rpl32-trnL* (± *trnL-trnF*) sequences were available. The ILD test indicated strong incongruence between the cpDNA-n data and ITS, ITS1, and ITS2 ($p = 0.001$ in all three cases). However, in this 27-sequence alignment, ITS1 and ITS2 were not significantly incongruent ($p = 0.24$).[17] Despite ITS/cpDNA-n incongruence, the data were combined for heuristic purposes in order to identify *points* of data compatibility/complementarity or lack thereof. (In)congruence was identified manually by undertaking five analyses: three combined ITS/cpDNA-n analyses (ITS/cpDNA-n, ITS1/cpDNA-n,

[17]This at least partially, if not substantially, reflects the smaller sample size. The number of possible trees (the sample size) in hyperhexahedric Buneman tree space increases exponentially with terminal number, in this case 27 versus 54. But the tree length distribution is related nonlinearly to this exponential curve, because it depends upon the idiosyncratic pattern of taxon similarity. So the reduction in the number of taxa also may have eliminated in an idiosyncratic manner branches that were inherently incongruent in optimized ITS1 and ITS2 trees. In any case, as discussed in Hershkovitz (2025b), the ILD test is a heuristic but often misleading indicator of data compatibility…*one way or the other*. In particular, in the case of a significant result, it does not identify the *cause* of the incongruence or its location(s) in hyperhexahedric Buneman tree space. And an insignificant result (up to $p = 1.0$) simply means something that we already know, viz. that trees with different MP *topologies* can have (arithmetically or statistically) the same *length*.

ITS2/cpDNA-n) and repeated bootstrap analysis of ITS and cpDNA-n separately using the common 27-sequence data in order to "compare apples with apples" and avoid cherry-picking.

Figure 9 shows the MP bootstrap results for the combined ITS/cpDNA-n data, with the BPs for the ITS1/cpDNA-n bootstrap also indicated along all branches and, for some branches, also the cpDNA-n and ITS2/cpDNA-n bootstraps. The ITS1/cpDNA-n bootstrap is less well-resolved overall and *mostly* less well-supported at particular nodes. But it is completely compatible with the ITS/cpDNA-n consensus. Incongruence of cpDNA-n and ITS2/cpDNA-n with the Figure 9 consensus is documented at four nodes. An additional node strongly supported in Figure 9 is incongruent with cpDNA-n alone and receives no significant support from ITS2/cpDNA-n. Figure 9, like Fig. 7, also shows some severe discrepancies between MP and ML bootstraps, e.g., < 50% for MP vs. 79% for ML.

For the present purposes, the most significant result of the combined analysis is the increased support for the sister relation between *N.* sect. *Nassauvia* and a clade comprising remaining *Nassauvia* and *Triptilion*, with *N.* sect. *Panargyrum* nested within the latter clade and with two origins of *N.* sect. *Triptilion* sequences. As noted above, this is what the cpDNA-n data alone shows, and it differs from what both ITS and, especially, ITS1 shows. The ITS/cpDNA-n bootstrap shows increased support for this relation compared to *rpl32-trnL* alone (Fig. 7). Interestingly, while support for this relation is reduced somewhat in the ITS1/cpDNA-n bootstrap, it still is rather high and, notably, *greater than* that for *rpl32-trnL* alone. This indicates that ITS1 may contain underlying support for the relations supported by ITS2 and cpDNA-n, but that this support is "masked" by convergent multiple substitutions, viz. high homoplasy. Not unexpectedly, support for this node is greater in the ITS2/cpDNA-n bootstrap.

Collectively, the data indicate that, while the ITS and cpDNA-n data indeed manifest incongruence, they seem to be more incongruent at *lower* taxonomic levels and not with respect to relations among the major clades. But there is an important caveat. The limited, but highly informative, ETS data (Fig. 5) seem to agree with ITS with respect to major clade relations, especially a sister-relation between *N.* sect. *Panargyrum* and *N.* sect. *Nassauvia*. ETS and ITS, of course, are different regions of the same locus. Adequate ETS sampling might demonstrate that the nuclear genome (proxied by rDNA ETS/ITS) and cpDNA-n indeed are incongruent, viz. have different histories.

Three examples of lower-level ITS/cpDNA-n incongruence are indicated at "middle" nodes in Fig. 9. An example is the relations among *N.* sect. *Triptilion* species. The combined data strongly support a clade comprising the annual species *N. berteroi* as sister to a clade comprising the perennial species. Monophyly of the remaining three annual species seems to be supported by the combined data and ITS1/cpDNA-n ML BPs, but not the MP BPs, and monophyly is disputed by both cpDNA-n and ITS2/cpDNA-n. Pending further analysis, I consider as unresolved the relations of these three species to each other and to the well-supported clade.

Other incongruencies occur at lower nodes but these mostly are not shown here. There is not much point at present. In particular, the separate data analyses show numerous examples of polyphyly among nominally conspecific genotypes. The low degree of sampling suggests that more is to be expected. Until these problems can be addressed with more exhaustive study, no broader resolution is possible. All that can be said is that incongruencies and polyphyly exist in this group. The one example illustrated in Fig. 9 involved the monophyly of *N.* sect. *Strongyloma*, strongly supported by the combined data and ITS1/cpDNA-n BPs, versus paraphyly, weakly supported by cpDNA-n, versus lack of resolution in the ITS2/cpDNA-n bootstrap.

d. Insights from network analyses. To better understand and appreciate the relations between the different data sets and their phylogenetic output, the ITS1, ITS2, cpDNA-n, and combined data from the 27-sequence alignment were analyzed using NeighborNet distance-based networks. This yields simplified

conceptually polyhexahedric networks, illustrated in Figures 10A–D, allowing simultaneous visualization of all possible planar quartet split decomposition distances.

Figures 10A–B show that, despite topological difference in their cladistic bootstrap consensuses, the ITS1 and ITS2 networks are geometrically very similar. This similarity explains the insignificant ILD incongruence score. The most important difference is the location of the root, viz. the *C. acerosus* branch. In either network, moving the root a couple of nodes yields a network with the *N.* sect. *Panargyrum* relations shown in the other. Otherwise, the ITS1 and ITS2 data disagree mainly at nodes not especially well-supported by the other network. However, a notable difference between ITS1 and ITS2 is the placement of *N.* sect. *Strongyloma*. ITS1 clusters these sequences with *N.* sections *Achilleae* and *Caloptilium* (cf. Fig. 3), while ITS2 clusters only the latter two somewhat remotely from *N.* sect. *Strongyloma* (cf. Fig. 4). This demonstrates that the support for the relation between *N. achilleae* and *N. lagascae* evident in Fig. 3 and reported elsewhere (Maraner2012, Jara2017a, Lavandero2024) derives only from ITS2 and not ITS1 (see Discussion).

In contrast, the relations of *N.* sect. *Panargyrum* in the cpDNA-n network (Fig. 10C) are very different. Here, this section "jumps over" the intervening *N. achillea* and *N.* sect. *Strongyloma* nodes and associates closely with *N.* sect. *Triptilion*. At the same time, it can be seen that the *N.* sect. *Panargyrum* samples do not form a well-defined common stem, and that its parallelograms intermix with those of *N.* sect. *Triptilion* parallelograms. Moreover, the *N.* sect. *Panargyrum* sequences seem to nest *among* the annual *N.* sect. *Triptilion* species (viz. in between *N. gibbosa* and the remainder). Both observations are consistent with cpDNA-n (only) bootstrap data in Fig. 9. The cpDNA-n data alone show no significant bootstrap support for monophyly of *either N.* sect. *Panargyrum* or *N.* sect. *Triptilion*. This owes mainly to the ambiguous relations of the *N. lagascae* sample, but also to ambiguity of the relations among annual *N.* sect. *Triptilion* species. At the same time, consistent with BPs from all data sets, Fig. 10C shows that these taxa together form a well-defined stem separated from *N.* sect. *Nassauvieae*.

A further notable feature of the cpDNA-n network is that its rooting point is essentially identical to that of the ITS1 data rather than the ITS2 data. This might seem peculiar, since it is ITS1, not ITS2, that shows *N.* sect. *Nassauvia* as sister to *N.* sect. *Panargyrum*. ITS2 *agrees* with the cpDNA-n data in showing the latter as sister to remaining *Nassauvia*. But comparison of the networks helps demonstrate that the major difference between the ITS1 and cpDNA-n data is not per se the position of the root, but the precise relations of *N.* sect. *Panargyrum*. This helps to explain why the ITS1/cpDNA-n consensus is congruent with the ITS/cpDNA-n bootstrap consensus, while the ITS2/cpDNA-n consensus is not, even though ITS2 and cpDNA-n agree with respect to cladistic relations of *N.* sect. *Panargyrum*, while ITS1 and cpDNA-n disagree.

Thus, the ITS network better resolves "monophyly" of *N.* sect. *Panargyrum*, but not so much its *relations*, because this depends only upon the visually "precarious" rooting. Meanwhile, the cpDNA-n are ambiguous regarding *monophyly* of *N.* sect. *Panargyrum*, but more decisively support its position relative to the root. Both monophyly and relations of *N.* sect. *Panargyrum* thus become unambiguous when the data are combined. This implies that, even though ITS and cpDNA-n separately disagree with respect *N.* sect. *Panargyrum* relations, they actually are complementary rather than incongruent on this point.

As in the cpDNA-n network, the combined data network also shows a discrete stem joining the *N. achillea* and *N.* sect. *Strongyloma* sequences. These sequences are merely proximal in the ITS networks. The ITS networks showed the *N. lagascae* sequence proximal to these, whereas the cpDNA-n and combined bootstraps and networks associate *N. lagascae* with *N.* sect. *Triptilion* sequences. The same principle applies: the ITS data are not so much incongruent as they are ambiguous. Finally, the position of the root in the combined data network results in distinct "stems" for each of the two major divisions of

Nassauvia. In the separate data set networks, the root position was biased, such that one but not the other of the divisions formed a stem.

Discussion

Comments on analytical methodology applied in the present analyses

The degree to which organismal phylogeny can be inferred from gene trees depends first and foremost (but not last nor least) upon the accuracy of the gene trees. I have discussed in detail (Hershkovitz, 2021a, 2025b, among others) aspects of the theoretical bases of molecular phylogenetic analysis and both the molecular and organismal evolutionary assumptions built into widely applied computational methods. This understanding is critical to diagnose not *if*, but *when* assumption violations are so severe as to bias results and conclusions. Unfortunately, apparently "scientifically sophisticated" laboratory and analytical methods often are simply copy-pasted and parroted in molecular systematics publications. This has been standard practice for decades.[18] Moreover, the "science" in such works is not that of the authors, but of *other* scientists. Indeed, the protocols are reasonably robust such that their robotic application can yield reliable results. But even then, this reflects chance rather than scientific rigor on the part of the authors. Other times, this approach can fail spectacularly (e.g., Jara-Arancio et al., 2017b [hereafter Jara2017b]; cf. Hershkovitz, 2024b).

There is no particular "protocol" for generating accurate trees. In my work, I apply (technically) unweighted MP and frequentist ML cladistic analysis and bootstraps.[19] Depending on the results, I also might explore distance and network methods (e.g., Fig. 10). I use MP and frequentist ML not simply because they are "there" in the software, but for a reason. They are complementary. Both MP and ML are vulnerable to error owing to violations of their assumptions. But they are vulnerable to violations of *different* assumptions, viz. along different vectors. For example, ML is more sensitive to among-site rate heterogeneity, because the substitution model is estimated over all sites. MP analyzes only parsimony informative sites. But MP is more vulnerable to branch attraction owing to high divergence and/or base compositional bias (e.g., Hershkovitz, 2024a, 2025b). But since standard ML methods erroneously model molecular evolution as a stochastic substitution process, it might be more vulnerable to errors owing to other processes and, perhaps most of all, more realistic but statistically intractable evolutionary *idiosyncraticity* (Hershkovitz, 2021a).

In any case, application of both methods is useful, because incongruent results at *any* node can betray both scalar and vector errors in underlying assumptions. In addition, most researchers simply perfunctorily "perform" ML/MP bootstraps and report the BPs without appreciating the diagnostic value of comparing BPs between methods and in separate versus combined analyses. Thus, my application of both methods is neither capricious nor merely perfunctory. It is an analytical *tool*.

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18 "*Unfortunately, phylogenetic analysis is frequently treated as a black box into which data are fed and out of which "The Tree" springs*." (Swofford et al., 1996: 407). The remark was intended to convey the reality that molecular systematic research (and its underlying components, phylogenetics, molecular evolution, and molecular biology) is highly complex theoretically, but that empirical applications of third-party molecular systematics laboratory and analytical tools and services indeed often are, effectively, mindless, if not superstitious.

19 I have discussed here and elsewhere (Hershkovitz, 2021a, 2025) my opinion of (pseudo-)Bayesian methods ("induction on steroids") and the reasons for their popularity. Besides these, I suspect that especially uneducated empirical researchers just like to watch the columns of *–ln* likelihoods marching up their computer screens, because it makes them imagine that they are MIT nuclear physicists, or perhaps commanders of soldiers marching down the Champs-Elysées.

But understanding of the theoretical basis of the method has other benefits. The program is stupid. It simply is making calculations based on the data that is entered. Understanding the calculations allows one to introduce "hacks" into the data if one demonstrates that these hacks will improve accuracy. The present cpDNA-n data provide an example (Fig. 1). As aligned, the eight bp motif in *rpl32-trnL* that characterizes only *N.* sect. *Nassauvia* sequences most likely did not arise via site-wise substitution. I suspect that prior analyses of these sequences aligned this motif as in Fig. 1, but without even noticing it. But since the alignments are not available, I cannot confirm this. One reasonable solution would have been to "separate" the motifs in the alignment, creating an indel, which could be scored as such. But I did not do this. By leaving the superimposed but likely not homologous positions, I effectively weighted it ca. eight-fold, viz. the number of additional "substitutions." Weighting is a philosophically justified and once commonly used but now nearly "forgotten" tool in MP analysis. Conceptually, it appeals to Bayesian *reasoning* (not pseudo-Bayesian analysis).

In this case, prior knowledge justified this weighting. First, the ITS data, which lack such a feature, showed *N.* sect. *Nassauvia* as highly divergent from remaining *Nassauvia*. Second, this is not a simple indel, but, effectively, the replacement of one eight bp motif by another at the same position. In my experience, this is exceedingly rare. If not for the prior knowledge of *N.* sect. *Nassauvia* divergence and the rarity of motif replacement, I might have treated these data differently. For ML analysis, I was not concerned that these spurious substitutions would affect substitution bias estimation. This is given the small number of spurious relative to "true" substitutions, as well as the relatively small to nil impact of small substitution bias estimates on phylogenetic results. Otherwise, the effect is analogous to that for MP. Adding eight false substitutions is a means of incorporating this highly unlikely transformation into an ML program that only allows substitution data. The same applies to the indel transitions: I effectively assigned to them the same likelihood as that for a single GT substitution. In this way, nonstandard information can be "hacked" into the analysis in a way that is both reasoned and reasonable from an evolutionary standpoint.

Relations among the principal lineages of *Nassauvia*

Figure 11 summarizes relations among the principal lineages of *Nassauvia* derived in the separate and combined analyses, and compares these with results generated in other studies, in particular Maraner2012, Vidal2012, and Jara2017a. These are the only analyses that sampled numerous *Nassauvia* species and at least one erstwhile *Triptilion* species. Other studies (Kim et al., 2002; Simpson2009; Lavandero2024) sampled one to a few species of these genera incidental to analyses of other taxa or taxonomic levels. Figures 11A–C summarize analyses of combined nuclear and cpDNA-n, while Figures 11D–I summarize analyses of ITS alone. *Nassauvia* sect *Mastigophorus* is ignored Fig. 11, because the scant available data indicates polyphyly the intersectional level. Also ignored are ITS sequences for *N.* sect. *Nassauvia* taxa that nest among *N.* sect. *Panargyrum*, since there are no cpDNA-n data to corroborate their relations. Finally, given the unexpected and/or incongruent results for several of the *sampled* nominal species, it must be *emphasized* that also ignored here are the 12 nominal *Nassauvia* species that remain *unsampled*.

Figure 11A illustrates the consensus, with the exception of a minor detail, for all of the present combined data analyses. The detail is that the cpDNA-n analyses showed the *N. achillea* sequences nested *within* the two *N.* sect. *Strongyloma* sequences, and the ITS2/cpDNA-n analyses thus showed this triplet as unresolved (see above).

Vidal2012's analysis of combined data from these same loci (Fig. 11B) is nearly congruent with the present combined data analysis. There are, however, several caveats: **(1)** Vidal2012 is a Masters' thesis; it is *published*, though normally it is not given the same weight as a journal publication; **(2)** given

that it is a Master's thesis, its qualities reflect not merely those of an inexperienced student, but those of the thesis directors and signatories, in this case P. Jara-Arancio ("cotutor"[20]) and M. Kalin Arroyo (principal tutor[21]); the latter two are effective authors; **(3)** while Vidal2012's combined data topology includes 19 *Nassauvia* samples (17 spp.), *trnL-trnF* sequences were available for only nine of these, and the more informative *rpl32-trnL* sequences for only six;[22] **(4)** Vidal2012's reported ITS, *trnL-trnF* and *rpl32-trnL* alignment lengths are much longer than those used here;[23] **(5)** Vidal2012 used only a distantly related Calyceraceae sequence as an outgroup, rather than more closely related Nassauvieae;[24] **(6)** Vidal2012's combined data analysis performed only (pseudo-)Bayesian phylogenetic estimation[25,26] and not an MP or ML bootstrap;[27] **(3)** even so, the topology indicates < 0.95 (pseudo-)Bayesian posterior

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[20] In Chile, a *secondary* or "support" tutor, who trains the student in some essential but incidental *component* of the research.

[21] The person who *primarily* trains the student in theory/methods, directs their research, and is *completely* responsible for its contents.

[22] At least this is what I infer from Vidal2012: Fig. 4, Table 4, and Anexo Figures 3–4.

[23] For ITS, *trnL-trnF*, and *rpl32-trnL*, Vidal2012 reported alignment lengths of, respectively, 747, 914, and 1059 bases. The lengths in the Figure 9 analysis here are 649, 813, 1005. Vidal2012 did not publish her alignment or, for that matter, even any of the sequences she generated, so I cannot identify the source of the difference. Most likely, she included additional flanking sequence, which tends to be low quality and not available for all sequences. Computational alignment artifacts also may expand alignments.

[24] Alignability of more variable regions of ITS and *rpl32-trnL* is poor to the point of randomness across this phylogenetic distance, especially when unguided by phylogenetic intermediates (Hershkovitz, 2024a). Almost certainly, this introduced both alignment errors and random "noise" to the phylogenetic analysis.

[25] Hershkovitz (2021a, 2025) explained the difference between Bayesian statistical analysis based on empirical observations (viz. data) that are presumed to be independent and identically distributed (IID) generally versus the application of Bayes formula to analyze pseudo-observations that are quasi-IID. The observations in (pseudo-)Bayesian phylogenetic analysis are repeated but not independent ML optimizations of the same data. Based on the thus-generated tree branch distributions, they yield an estimate of the probability of the occurrence of a tree branch in the following optimization attempt. This is analogous but not equivalent to estimating the probability of a subsequent coin flipping outcome based on the distribution of prior flips. The difference is that the flips are truly IID, and we do not know the outcome before flipping. We add that outcome to the data after it is determined. In contrast, in pseudo-Bayesian analysis, the data (in this case sequence data) are known beforehand and are *constant*, viz. we are not predicting the outcome of the next substitution. It is predicting only the accuracy of "scorekeeping" of outcomes. In any case, (pseudo-)Bayesian posterior probabilities do not provide a measure of "data support" in the sense as the bootstrap. They are a biased probability that the same tree branch will be estimated given the *same* Metropolis-coupled Markov chain Monte Carlo (MCMCMC$^{\sim\infty}$) algorithmic parameters and the same (presumptively but often not) IID empirical data and imperfect evolutionary model. Strictly speaking (I think) this is not (as I have carelessly parsed previously) the probability that a tree branch is actually "true," but rather the *confidence interval*, again, given the parameters and the *same* data. I need to verify this with a statistician.

[26] Unfortunately, the practice of performing *only* (pseudo-)Bayesian phylogenetic analysis still occurs (e.g., Svenson et al., 2025), mainly because many in the systematics community do not even *attempt* to understand the theoretical bases of the various methods, hence just copy-paste their analytical protocols from other publications. And, as I remarked in Hershkovitz (2021a), some researchers simply prefer the "aesthetics" of high PPs compared to usually much lower BPs. Even so, a "real" scientist at least would use multiple methods and at least would try to explain any significant differences in the results. Failing that, at least the use of multiple methods would permit knowledgeable *readers* to interpret the difference.

[27] The reason that Vidal2012 gave for not performing a MP bootstrap analysis is bizarre: "*Se realizó un análisis de evidencia total* [viz. combined ITS/cpDNA-n]…[only] *de inferencia bayesiana…No se realizó un análisis de parsimonia, pues los datos no son congruentes, lo cual fue determinado en la prueba de partición homogénea* [= ILD test]..." But seemingly obviously, incongruence also would then preclude a combined data analysis by *any* cladistic method. Possibly Vidal2012 believed that the locus-specific *substitution* models permitted in Bayesian analytical software would "correct" for *cladistic* incongruence. Besides that, in Hershkovitz (2025b), I explained the ILD test and quoted the well-known conclusion of Barker & Lutzoni (2002: 51) that phylogenetic analytical "*decisions based on the ILD would be misleading in a large proportion of cases.*" Notably, Jara2017a (including Vidal and Kalin Arroyp) did not mention the incongruence reported by Vidal2012 (*signed* by Jara-Arancio and

probability (PP) for the clade comprising *N. achillea* and *N.* sect. *Strongyloma* and that comprising *N. lagascae* and *N.* sect. *Panargyrum*. In spite of these shortcomings, it is remarkable that this analysis yielded essentially the same topology as the present combined data analyses. It certainly suggests that the present combined data results are robust to even serious violations of the analytical assumptions.

A notable result of Vidal2012 concerns the relations of the *N. axillaris* sequences. Katinas2008a published a combined ITS/*trnL-trnF* analysis that included four *Nassauvia* species, two from *N.* sect. *Strongyloma* (*N. axillaris* and *N. ulicina*) and two from *N.* sect. *Nassauvia*. The former two formed a clade sister to the latter two. This detail of Katinas2008a (but see below) is consistent with Fig. 11A–B, but not especially informative given the sparse sampling. Maraner2012 later published an ITS analysis of a broader *Nassauvia* sampling. Their *N. axillaris* sample mapped alongside *N. pygmaea* and *N. juniperina*, both classified by Cabrera (1982) in *N.* sect. *Mastigophorus*. No provenance for the *N. axillaris* specimen was given. The present ITS analysis shows both *N. pygmaea* and *N. juniperina* nested within *N.* sect. *Nassauvia*, hence the Maraner2012, result clearly conflicts with Katinas2008a. In fact, Maraner2012 noted that their result conflicted with morphological evidence *and also* their unpublished cpDNA-n data from the same sample. Nicola et al. (2014) concluded that the Maraner2012 results for *N. axillaris* owed to one or another error. Nicola2019 sampled ITS for 13 individuals from four *N. axillaris* collections spanning ca. 28–40S in Argentina (viz. east side of the Andes), and all mapped to *N.* sect. *Strongyloma*.

But Vidal2012's analysis includes ITS results for an independent collection of *N. axillaris* from a well-collected locality near Santiago at ca. 33S (viz. the *west* slope of the Andes). This sequence indeed coincides with *N. pygmaea*/*N. juniperina*, whereas the *trnL-trnF* sequence, as in Katinas2008a, maps to *N.* sect. *Strongyloma*. Vidal2012 cited Cabrera's (1982) monograph and both Katinas2008a and Maraner2012, but did not comment on this incongruency between her ITS and *trnL-trnF* trees, nor the taxonomically aberrant placement of *N. axillaris* in her ITS and combined data trees.

Nonetheless, if Vidal2012's results are correct, this perhaps validates the ITS results of Maraner2012. More importantly (again, if correct), the data suggest *intersectional* hybridization between *N. axillaris* and *N. pygmaea*. The latter species is distributed between 21–54S on the east side of the Andes. It is less common on the west side, but Rodríguez et al. (2018) indicated that it occurs in Chile (undoubtedly only at very high elevations) as far north as ca. 32S. But I am unaware of records from near Santiago. Vidal2012's combined ITS/cpDNA-n analysis maps *N. axillaris* to *N. pygmaea*/*N. juniperina*. But this is not surprising, because only the *trnL-trnF* sequence was available for *N. axillaris*, and this sequence varies little among *Nassauvia* species.

A further twist in this story emerges in Jara2017a (including Vidal; see below), whose combined data analysis based partially on DNA from several collections used by Vidal2012, but also database sequences from different specimens. In particular, Jara2017a used the ITS sequence from Maraner2012, the *trnL-trnF* sequence from Katinas2008a, and, unlike Vidal2012, also the more informative *rpl32-trnL* sequence from Nicola et al. (2014),[28] not available, of course, to Vidal2012. These cpDNA-n sequences

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Kalin Arroyo) and performed only a combined ITS/cpDNA-n analysis (with predictable consequent artifacts). Meanwhile, Jara2017b (also including Vidal) reported incongruence (and made available their separate locus trees) but dismissed its importance and also suggested that their combined data analysis effectively corrected for incongruence (see also Hershkovitz, 2024b, 2025b). The common thread here is Mary Kalin Arroyo, formally the principal tutor for both Vidal's and Jara-Arancio's molecular phylogenetics research and coauthor of Jara2017a–b and co-author/co-investigator of the grant funding Jara2017b (see Hershkovitz, 2024b).

[28] This, itself, is peculiar. From Jara2017a's data, it can be determined that they extracted and processed DNA from 18 of the 19 field collected specimens reported by Vidal2012. It is not clear whether or not these are new sequences or the same sequences used in Vidal2012. For species not sampled by Vidal2012, they extracted/processed DNA from herbarium material or, in some cases, used GenBank sequences. *Nassauvia axillaris* was the only case where

map to *N.* sect. *Strongyloma*. Notably, the present analysis of *rpl32-trnL alone* (Fig. 7) strongly supports the inclusion of the *N. axillaris* sequence in *N.* sect. *Strongyloma*, and, even more strongly, its *exclusion* from *N.* sect. *Nassauvia*. It is peculiar, then, that despite including *rpl32-trnL*, Jara2017a's phylogram *not only* maps *N. axillaris* to *N.* sect. *Nassauvia* clade with 1.0 (pseudo-)Bayesian PP, but *also* illustrates its scant *combined* data *divergence* from *N. juniperina*. I repeated the present combined ITS/cpDNA-n data analyses using the ITS and *rpl32-tnL* sequences cited by Jara2017a. This yielded a very different but ± predictable result.[29] Thus, I cannot explain Jara2017a's result, since the alignment is not available, and given Jara2017b (see Hershkovitz, 2024b) and Jara-Arancio et al. (2019; see Hershkovitz, 2025b), it probably is not worth the effort anyway. In any case, although Jara2017a also cited Cabrera (1982), Katinas2008a, and Maraner2012, they did not comment on their aberrant results for *N. axillaris*, and evidently did not perform the separate data analyses that would have revealed the incongruence.[30]

Otherwise, the Fig. 11A topology agrees with combined data topologies of other analyses that included only a few *Nassauvia/Triptilion* samples incidental to other analytical objectives. These include the analyses of Kim et al. (2002; 3 *Nassauvia* spp., 1 erstwhile *Triptilion* sp.), Simpson2009 (5 *Nassauvia* spp., 1 erstwhile *Triptilion* sp.), and Lavandero2024 (5 *Nassauvia* spp., 2 erstwhile *Triptilion* spp.). Figure 11A also agrees with the cpDNA-n analysis of Nicola et al. (2019; 10 *Nassauvia* spp.). It is not incompatible with the unresolved MP bootstrap tree of Katinas2008a (4 *Nassauvia* spp., 1 erstwhile *Triptilion* sp.), but it is incompatible with their ML phylogram topology. But there is not an ML bootstrap, which, I suspect, would have approximated the MP bootstrap result (see also below).

Figure 11C shows the combined data topology reported by Jara2017a, which added the problematic hypervariable ETS data (see above). Because of this, quite likely most of the informative variation in this analysis was random noise. Aggravating matters, this work reported only *combined* data results analyzed using *only* the (pseudo-)Bayesian method. The results differ significantly from the present and all previous analyses.[31] They show *N.* sect. *Strongyloma* as sister to *N.* sect. *Panargyrum*, and this clade, in turn, sister to *N.* sect. *Nassauvia*. In the present ITS and cpDNA-n analyses and also the Vidal2012 combined analysis, *N.* sect. *Strongyloma* always situates in a clade with at least *N. achillea* and otherwise with *N.* sect. *Triptilion* and *N. lagascae*. It only occurs in the same clade as *N.* sections *Panargyrum* and *Nassauvia* in analyses of the smaller of two ITS data sets analyzed by Vidal2012 (Figures 11G–H; discussed below). Figure 11C also differs from Figures 11A–B in showing *N.* sections *Panargyrum* and *Nassauvia* in the same clade. This is the relation suggested by analyses of ITS alone. But it also is strongly supported by ETS alone (Fig. 5). Possibly this explains the Fig. 11C result (but see below). However, if further (and better quality) ETS sampling demonstrates that the nuclear and cpDNA-

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they did not use a Vidal2012 specimen (Vidal & Kalin Arroyo SGO 16732). This is from near Santiago, where this species occurs fairly commonly along the (only) road (to the ski centers) heavily studied by Kalin Arroyo. Why Jara2017 did not use the Vidal2012 collection or an easily obtainable new collection is not clear. A new, independent ITS sequence will be *necessary* to resolve the conundrum of the existing sequences, and these results will be critical to advance understanding of *Nassauvia* evolution. I reported in Hershkovitz (2024b) a similar situation for Jara2017b: DNA was extracted/processed from herbarium specimens, even though many of those specimens did not amplify or yielded unusable sequences, and even for species readily available near Santiago.

[29] Hybrid taxa often appear in cladograms in a position intermediate between the parent terminals (McDade, 1997). That is the case here. In my MP and ML combined data analyses that included the *N. axillaris* sequences used by Jara2017a, the "Frankenstein" *N. axillaris* sequence emerged as a very long branch *sister* (95%/92% MP/ML BP) to the *clade* (100%/100% MP/ML BP) comprising *N.* sect. *Nassauvia*. In other words, this contrived sequence did not nest within *N.* sect. *Nassauvieae* (per ITS analyses and the Jara2017a combined data analysis), nor among *N.* sect. *Strongyloma* (per cpDNA analysis), but in a position *between* these.

[30] As noted above, Jara2017b did perform separate data analyses showing incongruence between ITS and cpDNA-n, but they (erroneously) dismissed its consequences for their combined data analysis (Hershkovitz, 2024b).

[31] The title of the present work is "new insights from old data." In particular, Jara2017a authors had at their disposal *all* of the data analyzed here.

n trees have different histories, this would render them irreconcilably incompatible for combined analysis. Jara2017a did not mention performing tests, statistical or otherwise, for data set incongruence.

But the *principal* conclusion of Jara2017b was that "[their] *study shows that the* [sister] *relationship between N. lagascae* and [*N. achilleae*] *is strongly supported* [by 1.0 PP]." They suggested that this conclusion was supported by the ITS analysis of Maraner2012, which sampled not *N. achilleae*, but *N. spinosa* (see Fig. 11I). The present ITS analysis found only weak support for this relationship (Fig. 9; cf. Fig 10a), and this appears to owe to ITS2 rather than ITS1 (Figures 3–4 and 10 A–B; cf. Figures 11D–F). But, according to the present analysis, a sister relation between *N. achillea* and *N. lagascae* is strongly refuted by all other loci in the combined data analysis of Jara2017a, viz. ETS (Fig. 5) and the two cpDNA-n loci, as well as in the present combined ITS/cpDNA analysis (Figures 9, 10A) and that of Vidal2012 (Fig. 10B). As noted, Jara2017a author Jara-Arancio and Kalin Arroyo tutored the work of Vidal2012, and Vidal is "middle author" of Jara2017a. So it is strange that Jara2017a claimed "strong support" for a result that clearly is refuted by, effectively, their *own* earlier analysis, which they did not cite. Without the Vidal218 or Jara2017a alignments, I cannot identify precisely the discrepancies between those works and the present work. But given the alignment and sequence problems reported here and also for Jara2017b (Hershkovitz, 2024b, 2025),[32] it seems almost certain that Jara2017a's results and conclusions are spurious.

As for ITS, both the MP and (pseudo-)Bayesian analyses of Vidal2012's smaller data set (Figures 11G–H), differ markedly from the present ITS results (Fig. 11D). Unlike all other analyses, these analyses show *Nassauvia* sensu Cabrera (1982) as monophyletic. However, this consensus result was supported by < 70% MP BP, though also 0.97 (pseudo-)Bayesian PP.[33] Notably, this tree was rooted using only a distantly related and poorly alignable Calyceraceae sequence (see below). Moreover, this result conflicts with that in Vidal2012's tree for a larger data set (Fig. 11I), which added GenBank sequences for several additional *Nassauvia* species and Asteraceae outgroups, and is more similar to the present results (Fig. 11H; cf. Fig. 12). There are topological differences between Figures 11D and 11H, but these are not well-supported in either the Vidal2012 or present analysis. Still, it is notable that *both* Figures 11H–I show erstwhile *Triptilion* as a *clade* (viz. *monophyletic*), whether as sister to *Nassauvia* (Fig. 11H) or nested therein. This result was not well-supported in the corresponding Vidal2012 analysis, but, at the same time, it was not convincingly refuted by the present analysis of ITS *alone*. Diphyly of the erstwhile *Triptilion* sequences is strongly supported only in the cpDNA-n and combined ITS/cpDNA-n analyses.

As indicated, Fig. 11H, and to a large degree Fig. 11D, is compatible with the earlier ITS results of Maraner2012. However, that analysis included only a single species of erstwhile *Triptilion* [*N.* (sect. *Triptilion*) *spinosa*]. Otherwise, the results are compatible with the ITS analyses of Lavandero2024 (5 *Nassauvia* spp., 2 erstwhile *Triptilion* spp.). All of these results are incompatible with the ITS tree of

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[32] Jara2017a shares technical similarities with Jara2017b; see Hershkovitz, 2024b, 2025b) that might have contributed to results different from those presented here. *Most* of the ITS, *trnL-trnF*, and *rpl32-trnL* sequences reported by Jara2017a are much better in quality than many reported in Jara2017b. But this seems to be a matter of luck that the specimens selected were higher quality, and that the "mindless" processing yielded better data. Clearly it is not because of the authors' technical knowledge and skill, as demonstrated by their ETS data. As in Jara2017b, Jara2017a's alignments seem to have included noisy flanking sequence. The alignment lengths they reported for ITS (808 bp), *trnL-trnF* (936 bp), and *rpl32-trnL* (1133 bp) are even longer than the excessively long alignments reported by Vidal2012 (see above). These lengths evidently reflect the lengths of the untrimmed raw sequences that they reported, which include substantial flanking sequence.[32] As noted above, they reported an ETS alignment length of 1486 bp included more than 1000 bp of both noisy and misinformative IGS repeats upstream of ETS, as well as several sequences whose ETS sequence itself was unusable. In two cases, there was not even ETS sequence in the document, only IGS sequence. For the present analyses, I trimmed ca. 120–150 bp from *each* of Jara2017a ITS, *trnL-trnF*, and *rpl32-trnL* sequences and > 900 bp of IGS from most of the ETS sequences.

[33] Another example of low BP/high PP that betrays the nature of (pseudo-)Bayesian analysis.

Simpson2009 (5 *Nassauvia* spp., 1 erstwhile *Triptilion* sp.). This tree is peculiar in that it shows the two *N.* sect. *Panargyrum* samples not as sister to the two *N.* sect. *Nassauvia* samples, but as *paraphyletic* with respect to the single [*N* sect.] *Triptilion* sample, *N. spinosa*. However, I repeated the Fig. 2 analysis with these sequences added and determined that the *N. spinosa* sequence reported in Simpson2009 is a *contaminant*; it is actually a poor quality *N. aculeata* sequence (*N.* sect. *Panargyrum*). It differs from the latter only by numerous autoapomorphic "substitutions" that alignment inspection reveal to be sequencing errors. Accounting for this, the results are compatible with all other ITS analyses, although they are not especially informative: the three *N.* sect. *Panargyrum* sequences are sister to the two *N.* sect. *Nassauvia* sequences, and these are sister to the only other sequence, *N. lagascae*.[34]

In summary, at present, Fig. 11A provides the most robust available *tentative* (nonetheless disputable) phylogeny of the major clades of *Nassauvia*. This phylogeny shows *N.* sect. *Nassauvia* as sister to a clade comprising all of the other taxa. The latter clade includes two origins of the erstwhile *Triptilion* rDNA and cpDNA-n sequences. But it remains unclear if Fig. 11A is the *true* historical phylogeny of the organisms or, alternatively, if it is an artifact of hybridization and/or gene lineage-sorting during the early divergence of this modern clade. In the results, I discussed evidence that the ITS and cpDNA-n trees are not incongruent, but are complementary, such that, variously, one resolves clade composition and the other clade relations. Part of the evidence for this is that the combined data bootstraps strongly support relations contradicted in the ITS data, especially ITS1. This is even though ITS is more informative than the cpDNA-n data. And the combined ITS2/cpDNA-n bootstrap supports the Fig. 11A tree more than either data set alone.

However, my reanalysis of the available ETS data suggests the possibility that the rDNA and cpDNA-n phylogenies are truly historically incongruent, with *N.* sect. *Panargyrum* species sharing a nuclear rDNA history with *N.* sect. *Nassauvia*, but a cpDNA-n history with the other *Nassauvia* sections. It might be significant, in this regard, that Nicola2019 mentioned that (scant) cytogenetic research revealed octaploidy in *N. aculeata* (*N.* sect. *Panargyrum*). They thus suggested that this may be consequent to "*ancient hybridization between herbaceous and shrubby Nassauvia*." This would seem consistent with the incongruent relations of *N.* sect. *Panargyrum*. But they failed to note that that their cited cytogenetic reference reported diploidy in the other examined *N.* sect. *Panargyrum* species. But the ploidy difference itself does not rule out ancient hybridization, as all examined *Nassauvia* species share the base diploid chromosome number of 11. In this regard, the evidence for intersectional hybridization between shrubby and herbaceous taxa involving *N. axillaris* may be significant, since this means that reproductive barriers supposed by Nicola2019 might never have evolved in the first place. In any case, more thorough (and accurate) ETS sampling may shed additional light on this conundrum.

Comments on the impact of outgroup *Nassauvia* relations on ingroup phylogenetic analysis

Hershkovitz (2024a) discussed how the inclusion of excessively divergent, hence poorly alignable, outgroup sequences can distort ingroup relations. Meaningful alignment can be estimated in some cases, but this requires base-by-base alignment analysis and significant understanding of molecular evolution. It is not something that an automated alignment program can achieve, because, however mathematically

[34] The other five ITS Simpson2009 sequences map, with (perhaps erroneous) differences, to nominally conspecific independently reported sequences. However, *all* of the *Calopappus*/*Nassauvia*/*Triptilion* sequences reported by Simpson2009 (incidentally, including Kalin Arroyo) share a spurious 24 bp deletion between positions 580–604 of the present alignment, while the *N. lagascae* sample has an additional spurious 23 bp deletion between positions 474–497. In Table 1, the Simpson2009 sequences include all those with the GenBank prefix "FJ." I did not examine Simpson2009's sequences for other taxa. Most likely, the deletions were introduced via an erroneous cut/paste operation during alignment editing.

sophisticated it might be, it is "stupid." It will output an alignment even when the input sequences are perfectly random. Excessively divergent sequences, are not, of course, *perfectly* random, but they can introduce artifacts sufficient to distort the alignment of closely related ingroup sequences. In particular, statistical support for relationships among taxa separated by "short" internal or external branches often hinges on one to a few base differences. In hyperinductive (pseudo-)Bayesian analysis, one base can be the difference between 0 and 1.0 PP.

The inclusion of excessively divergent and poorly alignable outgroup ITS sequences (see Hershkovitz, 2024a) likely affected Vidal2012's results. Evidence for this is that Vidal2012's expanded ITS tree (Fig. 12) shows both the family Calyceraceae and Asteraceae subf. Gochnatioideae as *sister taxa* nested *within* Asteraceae, and also Nassauvieae nested within Mutisieae (cf. Funk et al., 2009; see also Fu et al., 2016; Mandel et al., 2019). This seemingly conspicuous aberration was not noted by Vidal2012 or, evidently, her *effective* co-authors, Kalin Arroyo and Jara-Arancio, or by Jara2017a (including Vidal).

Furthermore, Vidal2012 used a similar but notably different and even more bizarre topology to estimate divergence dates for these taxa (Fig. 13). Although Vidal2012 acknowledged the then-oldest earliest age estimate for Asteraceae origins (~60my; Eocene), she nonetheless reported a "middle" Cretaceous age (95.7 my). This itself was an error: her chronogram (Fig. 13) shows this age not for the Asteraceae crown divergence, but for the Nassauvieae stem divergence. The chronogram actually shows the Asteraceae (erroneously including Calyceraceae) crown divergence at ca. 112 my, viz. late *Lower* Cretaceous. From this, it can be extrapolated, that "Asteraceae" *origins* would date to the *early* Lower Cretaceous, viz. ca. the age of the oldest angiosperm pollen fossils. Again, this conspicuous aberration was not noted by the effective co-authors, Kalin Arroyo or Jara-Arancio.

In this same vein, another aberration of Vidal2012's expanded ITS analyses (Figures 12[35]–13) involves the inclusion of 16 *Chaetanthera* Ruiz & Pav. (including *Oriastrum* Poepp. & Endl.; Hershkovitz, 2021a) sequences downloaded from Genbank. The species names themselves are omitted in Vidal2012's figures, which identify this portion of the topology only as the "*Chaetanthera* clade." However, this "clade" includes 18 rather than 16 terminals (cf. Maraner2012: Fig. 1). Since these sequences originated from my own work (Hershkovitz et al., 2006), I could determine that the extra two terminals correspond to *Mutisia* sequences (viz. the *outgroup* of *Chaetanthera*) that Vidal2012 neglected to mention, and again, Kalin Arroyo and Jara-Arancio overlooked. Vidal2012's chronogram thus shows the *Mutisia*-*Chaetanthera* (not *Chaetanthera*) divergence at 69.9 my, viz. late Cretaceous. As can be estimated using a ruler, the *Chaetanthera* crown divergence then dates to ca. 44 my. Using (obviously) the same data, Hershkovitz et al. (2006 [including Kalin Arroyo]; not cited by Vidal2012) estimated this age at 16.5 my. Quite a difference. In fact, the 16.5 my figure was highlighted in the article abstract because of its importance to the biogeographic conclusions. Even though Kalin Arroyo also coauthored *this* work (and in, fact, was the *principal* investigator), she later, without commentary, *effectively* coauthored Vidal2012's 44 my estimate. And only a few months later, she submitted another publication (Guerrero et al., 2013) that used the earlier 16.5 my estimate.

Both excessively divergent outgroups and excessive artifacts from poor alignment of poor quality sequence (especially ETS; see above) probably affected also the results of Jara2017a, and they may explain the 1.0 PP for the sister relation between *N. achillea* and *N. lagascae* (see above). As outgroups, Jara2017 used the *Nassauvia* sister *Calopappus*, which would influence most strongly the more conserved portions of the *Nassauvia* alignments. But successively more hypervariable regions of ITS and, especially, their IGS-ETS "data," likely would have been influenced by successively more distant and therefore successively less alignable outgroups. These included *Calorezia* Panero (Nassauvieae subtribe. Nassauviinae; Hershkovitz, 2025a) and *Pleocarphus* D.Don (N. subtribe Trixidiinae), which are

[35] Although topologically distinct, Vidal's figure is aesthetically identical to Fig. 1 of Maraner2012.

reasonably closely related, but then only the very distant *Nastanthus* Meyen (Calyceraceae; see above and Hershkovitz, 2024a).

Insights on *Nassauvia* evolution from interspecific relations within clades

The present work makes no pretense to resolve interspecific relations within *Nassauvia* clades. Both the interspecific and infraspecific sampling are woefully inadequate for that purpose, because the existing data already demonstrate considerable infraspecific polymorphism and incongruence between ITS and cpDNA-n trees. I did not, in any case, study the morphology/ecology of either the sampled individuals or thoroughly research morphology in genus in general, so I cannot make definitive judgments about morphological correspondence to molecular data at the interspecific level. I relied here especially on Freire1993. Nonetheless, analysis of the totality of the available data yields significant insights on *Nassauvia* evolution that are not evident from the earlier studies that analyzed only portions of the data.

Hershkovitz (2021a) suggested that infraspecific polymorphism of nominal "species barcode" loci was the rule rather than the exception in angiosperms. This "rule" emerges from practically all studies that sample more than one individual per nominal species (see, e.g., Nicola2019 and several studies cited therein, also Böhnert et al., 2019, and practically every multiply-sampled interspecific analysis of barcode or phylogenomic data published ever since). There should be a caveat added here. To *some* degree, apparent infraspecific polymorphism might reflect any or all of specimen misidentification, PCR-induced mutations during initial and later sequencing reactions, and misreading of sequence data. Jara2017b provide numerous examples of the first and last of these (Hershkovitz, 2024b, 2025b). The present analysis reveals examples of such errors in prior *Nassauvia* analyses, as detailed below.

Setting aside the caveats, quite often, the nominally infraspecific "species barcode" sequences appear not only as polymorphic, but also as *polyphyletic* in "species trees," while, at the same time, identical sequences often are shared *interspecifically*. This variability had been artificially masked by the historical tendency of molecular systematic studies to examine one individual per nominal species, perhaps combined with an essentially superstitious belief in the existence of species barcode loci.[36] In fact, both infraspecific polymorphism and interspecific genetic identity should not have been unexpected. In particular, the original molecular systematics bandwagon, viz. isozyme/allozyme approaches, generally focused on *infraspecific* variation at single/low-copy loci, and generally found it. Not surprisingly, therefore, this polymorphism emerges in phylogenomic analyses that sample multiple individuals (e.g. Böhnert et al., 2022).

Evolutionary *interpretation* of such polymorphisms and identities is another matter. Using a population genetics statistical model, Nicola2019 found that ITS variation in *N.* sect. *Strongyloma* fit

[36] Wolf et al. (2013) presented "proof" that infraspecific ITS polymorphism was consequent to compensatory base mutations that maintain ITS2 secondary structure. Thus, they argued that it is the secondary structure, rather than the sequence, that is species-specific, hence the "species barcode concept" was valid. This is a crock. As predicted, compensatory base mutations indeed occur, and these *presumably* are related to maintaining ITS2 function, though, if I recall, this has been studied only in yeasts. Meanwhile, ITS including, ITS2, is a relatively fast-evolving locus, and that is why it is used to reconstruct phylogenetic relations among closely related taxonomic species. Thus, compensatory base mutations *ought* to be correlated to a degree with taxonomic species limits. But that is as far as one can go. Compensatory base mutations cannot rescue the ITS species barcode concept from infraspecific variation in ITS1, which has an evolutionary origin and function different from ITS2 and apparently little functional secondary structure (Hershkovitz et al., 1999). And it cannot rescue other supposed species barcode loci, e.g., *rpl32-trnL*. And it cannot rescue ITS2 or any other locus from observed *interspecific* sequence *identity*. Everything we know about molecular evolution and variation at the species level leads to a prediction that species barcodes do not and *cannot* exist. This is not to say, of course, that such sequences have no taxonomic diagnostic value *at all*.

better a model of lineage sorting of persistent functional paralogs rather than cladistic divergence followed by subsequent interspecific gene flow. Basically, the ITS diversity manifested little rhyme or reason with respect to morphological or ecological parameters. But they also recognized that the overall statistical pattern did not rule out gene flow in particular cases, especially since the species are sympatric, and their general range in Patagonia has been ecologically unstable during the past several million years. The fact that the nucleotypes form two clades indeed might suggest a divergence of species bearing different *sets* of paralogs and later hybridization that allowed essentially all nucleotypes to occur in all species. While the cpDNA-n polymorphism also manifested no statistical pattern for hybridization, again, it cannot be ruled out in specific cases. Nicola2019 reported that the cpDNA-n morphs did manifest a latitudinal correlation, such that interspecific similarities at different latitudes might indeed represent introgression.

Shifting the focus to the other polytypic *Nassauvia* sections, Figures 3 and 6 show divergence and polyphyly of nominally infraspecific genotypes even more extreme than that demonstrated for *N.* sect. *Strongyloma*. This is especially true for ITS samples of *N.* sect. *Nassauvia* representing *N. dentata*, *N. magellanica*, *N. pygmaea*, and *N. revoluta* samples (but see below).We might reasonably wonder whether additional infraspecific sampling would reveal the same pattern as in *N.* sect. *Strongyloma*, such that Figures 3 and 6 are merely gene phylogenies substantially decoupled from phenotypic species phylogeny. Obviously, that question cannot be addressed here, and I cannot either verify here the taxonomic identity of the sampled specimens.

In theoretical terms, lineage sorting is a relatively simple genetic phenomenon. An *assumption* of lineage sorting resolves phylogeny in terms of compatibility and analyzes it with this or conceptually related coalescence methods. Meanwhile, interspecific hybridization is a complex genetic phenomenon whose immediate genotypic and phenotypic consequences are not at all or at least not entirely predictable. Longer term consequences following multiple generations are even less predictable, hence difficult or impossible reconstruct. Even in the first instance, interspecific hybridization presumes that the parents are different (and usually extinct) taxonomic species, and this distinction generally is subjective. For example, the *biological* species concept diagnoses species by their inability to interbreed. Hence, by *this* concept, strictly speaking, interspecific hybridization *cannot occur in the first place*.[37] In a phylogenetic context, inferred interspecific hybridization presumes that the *ancestors* already had differentiated as "taxonomic" species at the time of the crossing event. This, of course, is practically impossible to verify. This is part of the reason that hybridization and lineage sorting are difficult or impossible to distinguish.

Returning to the first instance, assuming the parents are different species, the offspring may be variously *taxonomically* phenotypically indistinguishable from one or the other parent. Because of epigenesis s. str. and s. lato (Vargas et al., 2020), the indistinguishable sort usually will resemble the female parent. Or the hybrid may be phenotypically intermediate, or, via transgressive segregation, notably phenotypically distinct from both parents. If the offspring are fertile and both distinct and reproductively isolated from the parents, hybrid *taxonomic* species can result. The consequences of hybridization at the DNA/genome level are more complex. In the case where F1 and later generations are taxonomically indistinguishable from one parent, it is possible that the effect of alleles from the other parent are innocuous or that they are "incompatible" in some way and therefore "silenced" or purged (see, e.g., Daugherty & Zanders, 2019) or that some portions of the genome are effectively resistant to introgressive recombination (Wong & Filatov, 2023[38]). But the effects of "species barcode" loci tend to

[37] This is ironic, since the "biological species concept" was popularized by the "biosystematic" school during the mid-20th Century. And this is the same school that *also* focused on interspecific hybridization. Go figure.

[38] This, in turn, seemingly would explain parentally inherent rather than post-hybridization-evolved subgenome dominance in allopolyploid hybrids (e.g., Liu & Wang, 2023 and many publications by these authors), but this

be innocuous with respect to phenotype and viability. This, along with their high copy number, can explain why these loci from one ancestral parental genome might persist even as the rest of that genome is eliminated. Following many generations, the result may be descendents of hybrids that are phenotypically and *mostly* genotypically indistinguishable from one ancestral parent but retain "species barcode" sequences of the other ancestral parent. Effectively, "species" evolution (viz. phenotype/behavior) transcends "gene" evolution.

Historical hybridization/introgression may be difficult or impossible to distinguish from lineage sorting of ancestral polymorphisms. Likewise, it may be difficult or impossible to know whether that ancestral polymorphism itself is consequent to mutation or hybridization. Hybridization/introgression is more likely when one or the other or both of the different loci from an individual map to divergent *tips* of their respective gene trees. In other words, lineage sorting is unlikely when one or the other or both of the sequences are very similar to sequences that themselves are related remotely. This is because it is highly unlikely that: **(1)** an ancestral allele subsequently reappears millions of years later in *only* two among many descendent species; and **(2)** these alleles retain their ancestral close similarity even though the rest of the genome is highly divergent. But as the "incongruent" sequences map to increasingly otherwise closely related taxa and/or as divergence between these sequences themselves increases, discrimination between hybridization/introgression and lineage sorting becomes impossible.

By analyzing all available sequences, the present analysis yields evidence of hybridization/introgression in some taxa not evident in prior analyses. The clearest examples involve *N. pygmaea* ("*N.* sect. *Mastigophorus*") and *N. magellanica* (*N.* sect. *Nassauvia*).Two independently obtained *N. pygmaea* ITS sequences (the "MG" and "MK" accessions) are identical (Fig. 3). Two independently obtained *N. magellanica* ITS sequences (also "MG" and "MK" accessions) are not only divergent, they polyphyletic, separated by two well-supported branches (Fig. 3). Likewise, both *N. magellanica* sequences are well-removed from the *N. pygmaea* sequences. However, the "MG" and "MK" *N. pygmaea rpl32-trnL* sequences are not identical. *One* of them is sister to the "MG" *N. magellanica* sequence (Figures 6–7), while the relations of the other is unresolved. In the combined ITS/cpDNA-n analysis (Fig. 9), the relations of the combined "MG" *N. pygmaea* sequences differ significantly from that suggested by either ITS or cpDNA-n alone. But this is an artifact of combining incongruent data.[39,40] The complexities above notwithstanding, the divergent relations of the "MG" ITS and cpDNA-n sequences seem to reflect hybridization rather than lineage sorting.

Another example of possible incongruence noted in the Results involves the relations among the annual species of *N.* sect. *Triptilion*, in particular the relations of *N. gibbosa* to the remainder. Interestingly, Vidal2012's combined ITS/cpDNA-n tree showed polymorphism and (albeit poorly supported) polyphyly of two *N. capillata* samples: "*T. capillatum* 1" sister to *N. cordifolium* and the "*T. capillatum* 2." sister to *N. gibbosa*. The polymorphism/polyphyly is shown also in her cpDNA-trees. But her ITS trees show only one *N. capillata* with no indication of collection 1 or 2. Maybe the ITS sequences were identical, but that was not reported. Vidal2012 did not comment on this polymorphism. Jara2017a's

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discussion is too digressive for this present work. I only bring it up to emphasize how understanding of these genome-level processes is critical to explanation of the relation between gene trees" and "species trees."

[39] The relations of *N. pygmaea* in the Jara2017a combined ITS/cpDNA-analysis are essentially the same as those in Fig. 9, viz. also artificial. But, as noted, Jara2017a did not report results for the separate data sets.

[40] I did not include the combined "MK" sequences in the Fig. 9 analysis, but I performed this analysis separately. This yielded 95%/96% MP/ML BP support for a sister relation between the combined "MG" and "MK." Otherwise, the relations of *N. pygmaea* remained ambiguously resolved, as in Fig. 9. This result reflects both overwhelming weight of the more informative and identical ITS sequences against the less informative and divergent *rpl32-trnL* sequences. There was no *trnL-trnF* sequence available for the "MK" accession individual.

combined analysis evidently reported results for only for the "*T. capillatum* 1" collection and likewise did not comment on the polymorphism/polyphyly they evident in Vidal2012.

At the same time, the present work also reveals cases where apparent polyphyly and incongruency owes to researcher error. An example is the apparent relations of four ITS sequences identified as *N. revoluta* (Fig. 2). All are different, but two of them ("MG" and one of the "EU" accessions) appear as sister, in turn sister to two *N. pinnigera* sequences. The other "EU" sequence is excluded from this clade (Fig. 3). Both "EU" accessions owe to Maraner2012, who, in fact, reported apparent *N. revoluta* polyphyly. A fourth sequence, the "EF" accession, is identical to the two *N. pygmaea* sequences, and highly divergent from the other three *N. revoluta* sequences.

Post-analytical reexamination of the data suggests that *N. revoluta* is not polyphyletic. The divergent "EU" sequence contains numerous substitutions/indels that are probably spurious, because they occur at positions otherwise 100% conserved in the ITS alignment. Thus, Jara2017a are hardly the only molecular phylogenetics researchers whose conclusions derive from (rather obviously) erroneous sequences. That leaves the *N. revoluta* "EF" sequence, which is identical to both of the *N. pygmaea* sequences. There is no corresponding *rpl32-trnL* sequence, so I did not include this sequence in the combined data analysis. But there is a corresponding *trnL-trnF* sequence, which is identical to the "MG" *N. pygmaea trnL-trnF* sequence. Given that two other independent *N. revoluta* ITS sequences are most closely related to each other and remotely related to the *N. pygmaea* sequences, this suggests the possibility that the "EF" sequences derive from an *N. pygmaea* individual misidentified as *N. revoluta*. While these species are easily distinguishable in the field, they appear similar superficially, and their differences probably are less evident in herbarium specimens. However, it is not *impossible* that a "true" *N. revoluta* individual was introgressed with both ITS and cpDNA from *N. pygmaea*. Meanwhile, until appropriate studies are undertaken, I consider inadequate the evidence for *N. revoluta* polyphyly.

Again, given the limited data, there is not much point in attempting here to phenomenologically dissect each instance of apparent polyphyly and congruence. But overall, there are data that support both lineage sorting and hybridization. For the more thoroughly sampled *N.* sect. *Strongyloma* species, Nicola2019s' data suggests that in some cases, interspecific lineage sorting has occurred. The rest of the genus is less well-sampled. But it is intriguing that, e.g., in the ITS tree (Fig. 3), out of nine of the total 13 species of *N.* sect. *Nassauvia* sampled 2X+ in this clade, eight are polymorphic, and five of these eight also appear as taxonomically polyphyletic. This degree of polyphyly might be expected under an interspecific lineage sorting scenario. But divergence patterns for particular species suggest hybridization. Most intriguing are the data for *N. axillaris*, which, if correct, document presumably recent intersectional hybridization. This means that, following many millions of years of diversification and geographic expansion (Nicola2019), interfertility between highly diverged lineages remains. And this raises the odds of hybridization scenarios in the case of other observed incongruencies.

Implications of the present results for phylogenetic *Nassauvia* taxonomy

Based on the present analysis and those of Hershkovitz (2024a) and Lavandero2024, Hershkovitz (2025a) constructed a new subtribal classification of Nassauvieae and revision of Cabrera's (1982) sectional classification of *Nassauvia*, in particular incorporating erstwhile *Triptilion*. These taxonomic changes are not capricious. As I noted in Hershkovitz (2024b), suprageneric names for established and otherwise taxonomically distinctive clades are preferable to inconsistent and otherwise uninformative informal taxon names like "Clade A, subclade III." For example, the subtribal Nassauvieae classification of Hershkovitz (2025a) circumscribes Nassauviinae and Polyachirinae, corresponding to divergent clades that are convergent for several characteristics (see Katinas et al., 2008a, b) differing from the other subtribes: **(i)** both include mainly herbaceous species; **(ii)** they are the only subtribes with annual species;

(iii) they are the only subtribes with *Oxyphyllum*-type pollen exines, which are found in all but one Polyachirinae species; **(iv)** both manifest evolution of pseudocephalia; and **(v)** they are the only subtribes distributed primarily from the Central Andes to the southern Andes (especially the western side) to extreme southern Patagonia (here extending eastward to the pampa). Thus, these subtribes represent a "model polyphylum" (cf. Donoghue & Edwards, 2019) of special interest to researchers studying evolution (and especially convergence) of southern Andean plants (and, of course, its relation to climate change, preserving our phylocracy, etc.).

This is not at all to say that taxonomic questions do not remain, in particular with respect to persistent phylogenetic irresolution, lack of data for several species, and strong support for certain incongruencies. Indeed, as discussed in the following section, it is complicated also by theoretical and philosophical problems, in particular, the relation between the *ontology* (for taxonomic purposes) of organisms and species and their molecular-level *genealogy*. Since this often challenges the very notion of species or even organismal "monophyly," it raises the question as to whether or not phylogenetic taxonomy itself is achievable or even *desirable* (cf. Stuessy, 2025). In particular, traditional phenetic taxonomy was replaced because it was considered to be biologically "misleading." Yet, phylogenetic taxonomy can be just as misleading. I focus here on empirical evidence for phylogenetic taxonomy and leave the theoretical and philosophical considerations for a later work.

a. Phylogenetic classification of erstwhile *Triptilion*. Based on the evidence presented here, Hershkovitz (2025a) reclassified *Triptilion* species as *Nassauvia* and divided the species among two sections. The present analysis leaves no doubt as to the nesting of at least *N.* sect. *Triptilion* sequences among *Nassauvia* sequences. This itself is not surprising, but all previous analyses manifest one or another shortcoming that might have raised doubts, whether inadequate sampling (Kim et al., 2002; Katinas2008a; Simpson2009; Lavandero2024), analysis of only ITS (Maraner2012), or technical/analytical flaws (Vidal2012; Jara2017a). The present analysis not only evaluates more data than previous analyses, but also provides the actual alignments (rather than just sequence accessions), so that the results can be scrutinized independently.

As for *N. achillea*, the ITS data remain somewhat ambiguous as to its relations (Fig. 3), whereas the *rpl32-trnL*/*trnL-trnF* data (Fig. 7) indicate it also is nested within *Nassauvia* and possibly even within *N.* sect. *Strongyloma*. However, support for the nesting of *N. achillea* increased when the ITS and cpDNA-n data were combined. At the same time, it cannot be ignored that the ETS (Fig. 5) and *ndhF* (Fig. 8) trees place *N. achillea* as sister to the *rest* of *Nassauvia*.

b. Phylogenetic evidence for the sectional classification of *Nassauvia*. Hershkovitz (2025a) otherwise maintained the sections of Cabrera (1982), but reclassified *N.* subg. *Strongyloma* (DC) Cabrera as *N.* sect. *Strongyloma* (DC) Hershk. Based on ITS analysis, Maraner2012 reported not only that *Nassauvia* (sensu Cabrera, etc.) is not monophyletic, but that *"neither of the two subgenera Nassauvia and Strongyloma is monophyletic, and none of the* [polytypic] *sections of subgenus Nassauvia is recovered as monophyletic*."[41,42] This conclusion derived partially from the placement of their *N. axillaris* (*N.* sect. *Strongyloma*) sequence among *N.* sect. *Panargyrum* sequences. Assuming that this result is correct and that some *N. axillaris* individuals are hybrids, it challenges both the meaning and, in many

[41] This conclusion was not mentioned by Vidal2012 or Jara2017, but these works did not attempt to reconcile their phylogenetic results with Cabrera's (1982) taxonomy.

[42] Freire1993's morphology-based cladogram already showed nonmonophyly of *N.* sections *Mastigophorus* and *Panargyrum*, as well as *Nassauvia* itself. Although the corresponding nodes in their cladogram are refuted by the molecular evidence, at least these results already had raised the *specter* of nonmonophyly before the advent of molecular evidence. At the same time, Freire1993's results cannot be dismissed entirely, since they provide additional clues regarding morphological versus "species barcode" evolution.

cases, the very existence of strict monophyly. In any case, the ITS of other individuals of *N. axillaris* indeed pertains to *N.* sect. *Strongyloma*, hence the hybrids were ignored for purposes of the phylogenetic sectional classification. Nonetheless, even ignoring *N. axillaris*, the Maraner2012 ITS tree and all subsequent ITS, cpDNA-n, and combined data trees still show *N.* subg. *Nassauvia* as nonmonophyletic. Thus, the only way to rescue monophyly of the section would be to alter the circumscriptions according to the phylogenetic evidence. But this is problematic, because the ITS and cpDNA-n disagree on any of the plausible circumscriptions.

As for monophyly of *N.* sect. *Strongyloma* itself, Nicola2019 reported that 4/5 species of this group contain persistent ITS paralogs that themselves pertain to two genotypic clades. They reported weak support for polyphyly of the paralog clades with respect to other possibly intervening/intruding *Nassauvia* taxa. The present ITS analysis cannot rule out this possibility, but the cpDNA-n data strongly supports monophyly of *N.* sect. *Strongyloma*. The latter evidence naturally overwhelms the former in the combined ITS/cpDNA-n analysis. It would be useful to sequence ETS for at least a portion of the individuals examined by Nicola2019, first to see if they manifest, as might be predicted, paralogy correlated with that of ITS. If so, the impact of phylogenetic relations of the two "total" rDNA paralog clades could be analyzed better. But Nicola2019 did not discuss monophyly of the complementary *N.* subg. *Nassauvia* sensu Cabrera (1982) or its sections. In fact, even though they sampled only one species per section and did not sample erstwhile *Triptilion*,[43] both their ITS and cpDNA-n trees already showed *N.* subg. *Nassauvia* sensu Cabrera (1982) as nonmonophyletic.[44]

Perhaps the most problematic section is *N.* sect. *Mastigophorus*. Based on morphology, Freire1993 found this to be nonmonophyletic; in fact, *paraphyletic* with respect to a clade comprising three subgenus *Nassauvia* sections plus *Calopappus*. Only two species (three individuals) have been sampled for "species barcode" loci, but only one species for both ITS and cpDNA-n. Both the gene trees place these individuals in *N.* sect. *Nassauvia*,[45] but the *N. pygmaea* sequences manifest "bizarre" incongruencies therein (see above). These species could be transferred to *N.* sect. *Nassauvia*, but this does not mean that *N.* sect. *Mastigophorus* belongs there. As noted, the inadequate but only available genomic evidence for relations of the Type of *N.* sect. *Mastigophorus, N. gaudichaudii*, places it at least close to if not within *N.* sect. *Panargyrum.* And this more or less concords with morphological evidence (Freire1993). Thus, the taxonomic fate of *N.* sect. *Mastigophorus* depends upon verification of the *N. gaudichaudii* specimen and, in any case, additional data. And, of course, there are no genomic data at all for the other three species classified by Cabrera (1982) in this section.

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[43] Nicola2019's omission of erstwhile *Triptilion* in both her analysis and discussion is peculiar. They cited not one, not two, not three, but *four* (of *six* then available) independently authored molecular analyses that showed erstwhile *Triptilion* as nested within *Nassauvia* (viz. Katinas2008a, Simpson2009, Maraner2012, Jara2017; cf. Kim et al., 2002; Vidal2012). They also cited three of numerous nonmolecular papers that indicated that erstwhile *Triptilion* (and not *Calopappus*) is (at least) literally or effectively sister to *Nassauvia* (Cabrera, 1982; Katinas1992; Freire1993). Yet, Nicola2019 did not sample an erstwhile *Triptilion*. As outgroups, they used only *Calopappus*, one other Nassauvieae, three Mutiseae, and three Barnadesioideae.

[44] Nicola2019 tacitly presumed that *N.* subg. *Nassauvia* and its sections sensu Cabrera (1982) were monophyletic, since they deliberately sampled one species per section. This tacit presumption was notwithstanding evidence for nonmonophyly in papers they cited (viz. Freire1993; Maraner2012; Jara2017a). Thus, it is not clear why they evidently assumed that including more sequences from *Nassauvia/Triptilion* species would *not* affect their results, while including, e.g., sequences from three Barnadesioideae, somehow *would*.

[45] Moreover, the ITS sequences of *N. pygmaea* cluster with that of *N. juniperina*, which corroborates Cabrera's (1982) intuition on the relations of the former at least to species he classified in *N.* sect. *Mastigophorus*. But, at the same time, the results from *ndhF* (see above) dispute monophyly of *N.* sect. *Mastigophorus* as a whole, unless the *N. gaudichaudii* specimen used in that analysis was grossly misidentified.

Sequences from the species that Cabrera (1982) classified in *N.* sect. *Panargyrum* are similar, and monophyly of the section is strongly supported in the combined ITS/cpDNA-n tree (Fig. 9) and its variants. But there are caveats. Phylogenetic support for the clade derives principally from the ITS data (Fig. 3) rather than the cpDNA-n data (Fig. 7; cf. 10C). But in the ITS tree, the section is *not* monophyletic: it includes sequences from two species of *N.* sect. *Nassauvia*. But there are no cpDNA-n sequences for these samples, hence there was no reason to include them in the combined data analysis. Morphologically, Freire1993 found that one of the species, *N. chubutensis*, is sister to remaining *N.* sect. *Nassauvia*, hence "anomalous" in this sense. But no such divergence was evident for the other species, *N. pulcherrima*. Given the apparent genomic "promiscuity" in this *Nassauvia*, I will not predict to which section the cpDNA-n sequences will pertain. Overall, the data suggest continued recognition of *N.* sect. *Panargyrum*, with the status of the interloping species remaining to be determined. Regardless, *strictly* speaking, Cabrera's (1982) diagnostic *concept* of *N.* sect. *Panargyrum* is not monophyletic.

Finally, from a genetic standpoint, monophyly of a clade *corresponding* to *N.* sect. *Nassauvia* is the clade best supported by *both* the ITS and cpDNA-n data. But even in this case, Cabrera's (1982) sectional concept is not monophyletic. Both the ITS and cpDNA-n sequences of one *N.* sect. *Mastigophorus* species, *N. pygmaea*, and *at least* the ITS sequence of another, *N. juniperina*, as well as the ITS sequences of two samples of an *N.* sect. *Strongyloma* species, *N. axillaris*, nest inside the *N.* sect. *Nassauvia* gene trees. It also is intriguing that the *N. axillaris* sequences are most similar to those of *N. pygmaea* and *N. juniperina*. Even more intriguing, both the ITS and cpDNA-n *N. pygmaea* sequences are not only polyphyletic, the relations of both samples are incongruent in the ITS and cpDNA-n trees. What is going on here? Nonetheless, it seems that *N.* sect. *Nassauvia* is best retained in the phylogenetic classification, with the anomalies resolved later.

Implications of the phylogenetic results for interpretation of the *evolution* of *Nassauvia*

Similar to Hershkovitz (2025b), the present analysis collated and analyzed together existing data that had been analyzed only partially in previous analyses, and also analyzed the data in a more consistent and rigorous way. This created a common platform that permitted refinement of prior phylogenetic estimates. The present analysis also identified several particular instances where sampling of additional individuals for these *same* loci would clarify certain persisting ambiguities. But, given the institutional structure of science, there is a low probability that additional *point* data, however useful, ever will be generated.[46]

At the same time, the analysis confirmed the naïveté of previous expectations that a few targeted gene variable sites in a genome would fully bifurcately resolve *organismal* phylogenetic relations (Hershkovitz, 2019). Likewise, it implicates a naïve (viz. superstitious) belief that phylogenomic approaches (viz., "more data") will bifurcately resolve organismal phylogeny better than targeted loci (Hershkovitz, 2021a, 2025b). This is not to say that additional targeted locus and/or phylogenomic data are useless. To the contrary, like all data, they are highly illuminating. But from an organismal phylogenetics perspective, perhaps the most illuminating result is the corroboration of targeted gene data

[46] Traditional morphological systematics is holistic; it advances more or less continuously, such that, e.g., new taxa are incorporated into existing classifications until such time that the next full revision is undertaken. Meanwhile, reductionist/experimental disciplines generally advance in discrete jumps, financed by spatiotemporally well-circumscribed projects that yield specified results in a specified time frame. Molecular systematics was financed according to the latter paradigm. All samples are collated processed ± simultaneously. Once a project is terminated, so is addition of data of the same sort, unless such data emerge incidental to *other* free-standing projects. That is the case for the *Nassauvia* data. The data are from multiple studies, one focusing on *Nassauvia*, one focusing on erstwhile *Triptilion*, and the others focusing on other taxa or taxonomic levels.

from many taxa that *already* have demonstrated thoroughly that gene trees are not species trees. However, phylogenetics/phylogenomics researchers, condition by "tree-thinking" dogma, still seem to focus only on parsimonious reconciliation of trees and ad hoc explanations for incongruencies, while not appreciating the *ontological* difference between gene trees and species trees and its significance. In other words, the paradigm "misses the forest for the (cladistic) trees" (Hershkovitz, 2019).

Hershkovitz (2021a, 2025b) went beyond the ubiquitous dogmatic approach of simply trying to reconcile gene trees and phenotypic species trees. I pursued an *explanation* for the discordance, viz. the *reason* for the interdependent yet evidently loose relationship between genes and species, hence the *inevitable* evolutionary consequences. As articulated in detail in the above and other publications, the explanation roots in the notion of biological autopoiesis (Maturana & Varela, 1972), viz. the concept of organisms as autopoietic systems, viz. self-organized and maintained and reproduced by hierarchically arranged systemic processes. In this concept, DNA is merely component, viz. historical "baggage" that, however *limiting* to the phenotypic *behavior* of organisms, does not *determine* it. Phenotypic behavior is a *sui generis* and *unpredictable* process of epigenesis sensu Vargas et al. (2020).

Phenotypic evolution thus is determined not by changes in DNA or environments, but by the conservation of changes in the adaptive *behavior* of the organisms within their milieu. *Evolution*, thus, is a process of change as *both* organisms and their genomes adaptively[47] "drift" in their milieu (Maturana & Mpodozis, 2000; Mpodozis, 2022). Genome and organismal evolution are associated, but cladogenesis and anagenesis among genomic loci and between the genome and organismal phenotype are not strictly correlated.[48] This mainstream phylogenetics assumption, or at least its *idealization*, precisely misleads mainstream phylogenetics interpretation of *evolution*.

Consequently, evolution necessarily is *idiosyncratic* (~ ∞-order) rather than stochastic (1º-order) Markov process (Hershkovitz, 2021a; cf. 2019, 2021b, 2025b). The significance of this cannot be understated, because mainstream evolutionary/phylogenetic analysis generally is *statistical*, which *presumes* stochasticity. This is not at all to say that statistical evolutionary analysis per se cannot be "correct" and is therefore invalid. That would invalidate results even of the present analysis. Rather, statistical analysis is a *heuristic* tool. Its analytical power derives not from the inherent assumption that analysis of increasing data will converge on the "truth," but rather that the "truth" might emerge via *comparative analysis* of statistical analytical failures.

Hershkovitz (2025b) described a case that demonstrated the *operational* relevance of these theoretical considerations. This involved inference of the evolution of the annual life history in *Leucheria* Lag. (sensu Hershkovitz, 2024b–c, 2025b). Phylogenetic analysis of "species barcode" loci suggested multiple origins of the annual habit (Jara2017b; Hershkovitz 2024b, 2025b; Lavandero2024). But

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[47] I use the term "adaptive" in the sense of Maturana & Mpodozis (2000), viz. if it is *alive*, then de facto it is *adapted*, such that *adaptive evolution* refers to nothing more than evolution that permits lineage continuity. This contrasts the notion of adaptation towards increased "fitness," both in the Darwinian sense (viz. an idealized functional optimum) or the neo-Darwinian sense (viz. relative reproduction rate).

[48] Hershkovitz (2006) noted the lack of expected cladogenetic and anagenetic diversification of nominal species barcode loci within certain Montiaceae genera, notably in *Cistanthe* Spach. In particular, nucleotypes and cpDNA haplotypes were shared not merely among phenotypically similar species (as in Nicola2019), but highly divergent ones, as well: small annuals to alpine perennials to small pachycaul trees. This was unfortunate, because this unexpected lack of divergence resulted in a lack of *anticipated* publications of resolved generic phylogenies and phylogenetic comparative analyses. And this made me appear to be "incompetent," viz. *unable* to resolve phylogenies. This was the last nail in my academic coffin. Trying to make the best of it, Hershkovitz (2006) suggested that the phenotypically divergent lineages might be older than the molecular marker divergences would suggest, but that one or another mechanism resulted in molecular markers "surfing" the tips of the trees. This work was done in my (naïve) "pre-Maturana" ontogenetic phase. Now I appreciate that I actually was "on" to something.

Hershkovitz (2025b) challenged the assumption that DNA sequence evolution faithfully tracks phenotypic evolution. The annual life history might have evolved once and persisted through subsequent reticulating DNA sequence evolution. The same logic can be applied in the case of the life form/history of erstwhile *Triptilion* species. The present DNA sequence phylogeny suggests that these arose twice. But, once again, the *Triptilion* phenotypes *might* have originated once and persisted through reticulated genomic evolution. This does not mean that this actually happened, but rather that it is a reasonable possibility in the theoretical framework described here. Moreover, this explanation is consistent with evidence at the chromosomal scale (e.g., Daugherty & Zanders, 2019;Wong & Filatov, 2023). Rather than dogmatically accepting the multiple origins suggested by the DNA sequence trees, this alternative hypothesis can be tested with further analysis.

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Supplemental files. The separate ETS, ITS, cpDNA-n, and *ndhF* data sets in NEXUS format for this work is available at: TO BE ADDED.

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Table 1. GenBank sequence accessions for *Nassauvia*, *Triptilion*, and *Calopappus* ETS, ITS, *trnL-trnF*, *rpl32-trnL*, and *ndhF* sequences. This list excludes sequences reported by Nicola2019 for *N.* sect. *Strongyloma* except for those used in the present analyses. Otherwise it includes all available sequences. Sequences used in the individual locus analyses are indicated in the figures. Sequences used in the combined ITS/cpDNA-n analysis are underlined. All original sequences were trimmed on one or both ends according to the alignment. Comments on particular accessions (mostly those *excluded* from the present analyses) are described in the table footnotes. Note that the arrangement of sequences in the *rows* is arbitrary; it does not mean that those sequences pertain to the same individual or analysis.

GENUS	SPECIES	AUTHOR	ETS	ITS	*trnL-trnF*	*rpl32-trnL*	*ndhF*
Calopappus	*acerosus*	Meyen	MG581281 MN582025	KY010360 FJ979685	KY223737	KY223786 FJ979735	KM192101
Nassauvia	*achillea*	(DC) Hershk.	MG432218 MN582044[1]	MG432152 MN582016	MG432174 KT345165[2]	MG432196	KT345069
Nassauvia	*aculeata*	Poepp. & Endl.	MG432224	MG432159 EU239282 FJ9795688[3]	MG432181 KY223738	MG432203 KY223787 MK786842 FJ979737[4]	
Nassauvia	*argentea*	Phil.	MG432225	MG432160 EU239263	MG432182	MG432204	
Nassauvia	*argyrophylla*	Speg. ex Hosseus		EU239264	EF530292		
Nassauvia	*axillaris*	D.Don		EU239273 MK786963 MK786976 EF530232[5]	EF530278	KM978459	
Nassauvia	*benaventii*	(J.Rémy) Hershk.		MG432153	MG432175	MG432197	
Nassauvia	*berteroi*	(Phil.) Hershk.	MG432219	MG432154	MG432176	MG432198	
Nassauvia	*capillata*	D.Don	MG432220	MG432155 EF5302222[7]	MG432177[6] EF530268	MG432199	
Nassauvia	*chubutensis*	Speg.		EU239268			
Nassauvia	*cordifolia*	(Lag.) Hershk.	MG432221	MG432156	MG432178	MG432200	
Nassauvia	*cumingii*	Hook. & Arn.	MG432226[8]	MG432161 EU239265	MG432183	MG432205	
Nassauvia	*darwinii*	O.Hoffm. & Dusén ex Dusén		EU239283		MG581284 MK786843	
Nassauvia	*dentata*	Griseb.	MG432227[8]	MG432162 AF422128 EU239269	MG432184	MG432206	
Nassauvia	*digitata*	Wedd.	MG432228[8]	MG432163 EU239287 FJ979690[9]	MG432185	MG432207 FJ979690	AF233824
Nassauvia	*dusenii*	O.Hoffm.	MG432229	MG432164 EU239266	MG432186	MG432208	
Nassauvia	*fuegiana*	(Speg.) Cabrera		EU239272 MK786984		KM978460	
Nassauvia	*gaudichaudii*	Cass.					L39405
Nassauvia	*gibbosa*	(J.Rémy) Hershk.	MG432222	MG432157	MG432179	MG432201	
Nassauvia	*glomerata*	Wedd.	MG432230	MG432165 EU238270	MG432187	MG432209	
Nassauvia	*glomerulosa*	D.Don		EU239280		KM978465	
Nassauvia	*heterophylla*	(Phil.) Reiche		FJ979687[10]		FJ979737[11]	
Nassauvia	*juniperina*	Skottsb.		EU239271			
Nassauvia	*lagascae*	(D.Don) F.Meigen	MG432231	MG432166 EU239288 FJ979686[12]	MG432188	MG432210 FJ979736 MK786844	AF233826
Nassauvia	*looseri*	Cabrera	MG432232	MG432167	MG432189	MG432211	

Table 1, continued.

GENUS	SPECIES	AUTHOR	ETS	ITS	*trnL-trnF*	*rpl32-trnL*	*ndhF*
				EU239275	EU547662		
Nassauvia	*maeviae*	Cabrera				MK786936	
Nassauvia	*magellanica*	J.F.Gmel.	MG432233[8]	MG432168	MG432190	MG432212	
				MK786957		MK786845	
Nassauvia	*pinnigera*	D.Don	MG432234	MG432169	MG432191	MG432213	
				EU239284		FJ979741[13]	
				FJ979691[14]			
Nassauvia	*planifolia*	Wedd.		EU239281			
Nassauvia	*pulcherrima*	Cabrera		EU239285			
Nassauvia	*pygmaea*	Hook.f.	MG432235	MG432170	MG432192	MG432214	EU385186
				MK786958	EU385092	MK786846	
				EU239267[15]			
Nassauvia	*pyramidalis*	Meyen	MG432236	MG432171	MG432193	MG432215	
				EU239274	EU547663		
Nassauvia	*revoluta*	D.Don	MG432237	MG432172	MG432194	MG432216	
				EU239277	EF530299[16]		
				EU239278[17]			
				EF530253[18]			
Nassauvia	*spinosa*	(Ruiz & Pav.) D.Don	MG432223	MG432158	MG432180	MG432202	AF233825
				FJ979689[19]	KY223739	KY223788	
				KY010302		FJ979739	
Nassauvia	*ulicina*	Macloskie		EU239279	EF530291	KM978491	
				EF530245[20]			
Nassauvia	*uniflora*	Hauman	MG432238[8]	MG432173	MG432195	MG432217	

Footnotes:

[1] A single substitution different from MG432218 (*N. achillea*); not used in the analysis because all for sequences from the same individual were available for the other sequence.

[2] Differs from MG432152 (*N. achillea*) by two substitutions and a two-base indel, but nonetheless maps to the other individual and no other species; not used in the analysis because all for sequences from the same individual were available for the other sequence.

[3] Similar to other *N. aculeata* sequences, but numerous Y/R base calls; excluded from the analyses.

[4] Same sequence as MG432211 (*N. looseri*), FJ979737 (*N. heterophylla*), and MG432203 and KY223787 (both *N. aculeata*); excluded from the analyses.

[5] *N. axillaris* (*N.* sect. *Strongyloma*) sequence used in Maraner2012 and Jara2017a; excluded from the analyses because it maps to *N.* sect. *Nassauvia* (see text).

[6] "Garbage" sequence; excluded from the analysis.

[7] Differs from MG432155 (*N. capillata*) by one substitution and one single-base indel; not used in the analysis because all for sequences from the same individual were available for the other sequence.

[8] "Garbage" sequences excluded from the analysis, variously with numerous ambiguities, evidently spurious substitutions/indels, or only IGS sequence completely lacking ETS sequence.

[9] Same sequence as MG432164 and EU239226 (both *N. digitata*); excluded from the analysis.

[10] Same sequence as MG432173 (*N. uniflora*) and EU239275 (*N. looseri*); excluded from the analyses.

[11] Same sequence as MG432211 (*N. looseri*), and MG432203, KY223787, and FJ979738 (all *N. aculeata*).

[12] Same sequence as MG432208 (*N. pinnigera*); excluded from the analyses.

[12] Similar to and monophyletic with MG432166 and EU239288 (both *N. lagascae*), but differs by evidently spurious substitutions and the 23 bp deletion shared by all "FJ" sequence accessions sequences (from Simpson2009) listed here; excluded from the analysis.

[14] Same as EU239284 (*N. pinnigera*); excluded from the analyses.

[15] "Garbage" sequence excluded from the analyses; numerous evidently spurious substitutions/indels.

[16] Not included in the combined ITS-cpDNA-n analysis, because no *rpl32-trnL* sequence is available for this plant sample. Nonetheless, both the ITS and *trnL-trnF* sequences map to *N. pygmaea*, hence possibly misidentified as *N. revoluta* (see text).

[17] Included in the ITS analysis, but contains several evidently spurious substations/indels, probably explaining its phylogenetic divergence from other *N. revoluta* sequences in Fig. 2.

[18] Included in the ITS analysis, but both the ITS and *trnL-trnF* sequences from this individual map to *N. pygmaea*, hence possibly misidentified as *N. revoluta* (see text).

[19] Bizarre sequence. The ITS2 is *identical* to MG432153 (*N. benaventii*) and distinct from the other *N. spinosa* ITS2 sequences, but most of the ITS1 maps to *N.* sect. *Panargyrum*. Thus, the whole ITS maps to the latter. Possibly a cut/paste

Table 1, continued.

GENUS	SPECIES	AUTHOR	ETS	ITS	*trnL-trnF*	*rpl32-trnL*	*ndhF*
artifact in the original Simpson2009 data matrix. Excluded from the analysis.							
[20] Correctly maps to *N.* sect. *Strongyloma* but not used in the analysis.							

Figure captions

Figure 1. Portion of the present *rpl32-trnL* sequence alignment showing an eight bp motif at positions 321–328 that discriminate between sequences of *N.* sect. *Nassauvia* and all other sequences.

Figure 2. One of 540 MP phylograms (RC [Farris, 1989] = 0.60; homoplasy index (HI [1 – HER; Archie, 1989] = 0.32) for the 54-sequence ITS alignment. *Apparent* nonmonophyly of sequences associated with different samples of the same nominal species is denoted with an asterisk. The Discussion suggests that polyphyly of the *N. revoluta* sequences owes to sequencing error and specimen misidentification. Three additional sequences not or only trivially different from other sequences are not shown (cf. Table 1): *N. digitata* (FJ979690), identical to the other *N. digitata* sequences; *N. heterophylla* (FJ979687), identical to the *N. uniflora* sequence; *N. capillata* (EF530222), trivially different from the analyzed *N. capillata* sequence.

Figure 3. MP/ML bootstrap consensus tree for the 38-sequence ITS alignment condensed from the Fig. 2 analysis. Bootstrap results for ITS1 are indicated to the right or below the total ITS results. Clade A includes *N.* sect. *Panargyrum*. A small asterisk indicates 100% BP, while a hyphen indicates < 50%. Polyphyly of sequences associated with different samples of the same nominal species is denoted with a large asterisk. (Sorry…I did not realize that I used asterices twice, and I am not going to go back and change the figures. Nobody is paying me for this.). **Footnotes:** Omitted sequences relative to Fig. 2, [1]same sequence as *N. looseri* (EU239275) and *N. heterophylla* (FJ979687); [2]same sequence as *N. pygmaea* (MK786958), *N. revoluta* (EF530253); [3]same sequence as *N. cumingii* (EU239265), *N. digitata* (MG432163, EU239287, FJ979690), *N. pyramidalis* (EU239274), *N. planifolia* (EU239281); [4]same sequence as *N. ulicina* (EU239279); [5]same sequence as *N. spinosa* (KY010362); otherwise omitted are sequences different from but monophyletic with (one of) the conspecific sequences: *C. acerosus* (FJ979685), *N. fuegiana* (EU239272), *N. lagascae* (EU239288), *N. pinnigera* (EU239284), *N. revoluta* (MG432172).

Figure 4. MP/ML bootstrap consensus tree for 38 ITS2 sequences. Clade A' includes Clade B of Fig. 3 plus *N.* sect. *Panargyrum*. A small asterisk indicates 100% BP, while a hyphen indicates < 50%. Polyphyly of sequences associated with different samples of the same nominal species is denoted with a large asterisk. **Footnotes:** Omitted sequences relative to Fig. 2, [1]same sequence as *N. looseri* (EU239275) and *N. heterophylla* (FJ979687); [2]same sequence as *N. pygmaea* (MK786958), *N. revoluta* (EF530253); [3]same sequence as *N. cumingii* (EU239265), *N. digitata* (MG432163, EU239287, FJ979690), *N. pyramidalis* (EU239274), *N. planifolia* (EU239281); [4]same sequence as *N. ulicina* (EU239279); [5]same sequence as *N. spinosa* (KY010362); [6]sequences different from but monophyletic with (one of) the conspecific sequences: *C. acerosus* (FJ979685), *N. fuegiana* (EU239272), *N. lagascae* (EU239288), *N. pinnigera* (EU239284), *N. revoluta* (MG432172).

Figure 5. MP/ML bootstrap tree for 17 ETS sequences. A small asterisk indicates 100% BP, while a hyphen indicates < 50%.

Figure 6. One of 9716 MP phylograms (RC = 0.77; HI = 0.17) for the 38-sequence *rpl32-trnL* alignment. Polyphyly of sequences associated with different samples of the same nominal species is denoted with an asterisk. The symbol Ⓐ refers to infraspecific polymorphism reported by Nicola2019. Two additional sequences identical to other sequences are not shown (cf. Table 1): *N. aculeata* (FJ979737), identical to MG432203, etc.); *N. pinnigera* (FJ979741), identical to MG432213.

Figure 7. MP/ML bootstrap tree for 31 *rpl32-trnL* sequences from Fig. 6. For two branches, black-background text indicates MP/ML BPs for the equivalent branch for combined *trnL-trnF*/*rpl32-trnL* data in the 27-sequence combined ITS/cpDNA-n data set (cf. Fig. 9). A small asterisk indicates 100% BP, while a hyphen indicates < 50%. Polyphyly of sequences associated with different samples of the same nominal species is denoted with a large asterisk. The symbol Ⓐ refers to infraspecific polymorphism reported by Nicola2019. **Footnotes:** Omitted sequences relative to Fig. 6, [1]same as *N. aculeata* (MG432203, KY223787, FJ979737); [2]same as *N. spinosa* (KY223788); [3]same as *C. acerosum* (FJ979735). Also omitted is *N. pinnigera* (FJ979741), identical to *N. pinnigera* (MG432213).

Figure 8. ML phylogram and MP/ML bootstrap for seven *ndhF* sequences.

Figure 9. MP/ML bootstrap consensus tree for combined ITS/cpDNA-N data (27 ITS and *rpl32-trnL* sequences and 24 *trnL-trnF* sequences. The MP/ML BPs for the combined ITS/cpDNA-n data are indicated above the branch in black text. Below the branch in black-background text are MP/ML BPs for ITS1/cpDNA-n data, and below this in blue-background text for selected ITS2/cpDNA-data. Red-background text show selected cpDNA-n MP/ML BPs. A small asterisk indicates 100% BP, a hyphen < 50%, and X a branch not present in the corresponding bootstrap consensus. **Footnote:** [1]denotes samples lacking a *trnL-trnF* sequence.

Figure 10. SplitsTree split decomposition networks. **A.** ITS1 network. "A" and "B" correspond to nodes A and B in the Fig. 3 cladogram, but in the network, they co-localize. **B.** ITS2 network. "A'" corresponds to node A' in Fig. 4. Note that node "A'" can be converted to "A" and "B" of Fig. 10A simply by sliding the *C. acerosus* branch two nodes towards the right. **C.** cpDNA-n network. The long branches of *N. darwinii*, *N. fuegiana*, and *N. magellanica* are artifacts of the missing *trnL-trnF* sequences. The other long branches reflect sequence divergence and are not artifacts. **D.** Combined ITS/cpDNA-n network. The long branches of *N. darwinii*, *N. fuegiana*, and *N. magellanica* are artifacts of the missing *trnL-trnF* sequences. The other long branches reflect sequence divergence and are not artifacts. Possibly I could have eliminated the branch artifacts at a preferences step, but these diagrams take a long time to optimize and label in the SplitsTree graphic editor, and the edits cannot be saved for subsequent modification.

Figure 11. Summary cladograms of sectional level phylogeny extracted from the present bootstrap consensuses for the indicated data sets and those of earlier workers. **Footnotes:** [1]The cpDNA-n consensus nests (with low BPs) *N. achillea* within *N.* sect. *Strongyloma*. [2]For *Nassauvia*, this analysis included 17 ITS, nine *trnL-trnF*, and six *rpl32-trnL* sequences. [3]For *Nassauvia*, this analysis included ITS sequences from 15 species. BE refers to (pseudo-)Bayesian analysis. For *Nassauvia*, this analysis included ITS sequences from 15 species. [4]For *Nassauvia*, this analysis included 24 ITS sequences from 23 species. [5]This analysis included only one erstwhile *Triptilion* sequence, *N.* (sect. *Triptilion*) *spinosa*.

Figure 12. A. Vidal2012: Fig. 7, consensuses for MP bootstrap and (pseudo-)Bayesian analysis of an expanded sampling of ITS sequences. The *Mutisia* branches were not identified in Vidal2012's figure and were added here. Branches indicate BE PPs followed by MP BPs. 1.0 PPs and 100% BPs are indicated with an asterisk. **B.** Cladogram of canonical suprageneric relations derived from numerous analyses. **C.** Cladogram of suprageneric relations derived from Vidal2012: Fig. 7.

Figure 13. A. Adapted from Vidal2012: Fig. 9A, a molecular chronogram showing Vidal2012's age estimates for certain clades. The *Mutisia* branches were not identified in Vidal2012's figure and were added here. Note that the topology contains numerous differences from the Fig. 12 topology. I in red-background text added the age estimate for the *Chaetanthera/Oriastrum* crown divergence. This was estimated here using a ruler. **B.** Cladogram of canonical suprageneric relations derived from numerous analyses. **C.** Cladogram of suprageneric relations derived from Vidal2012: Fig. 9A.

Figure 1

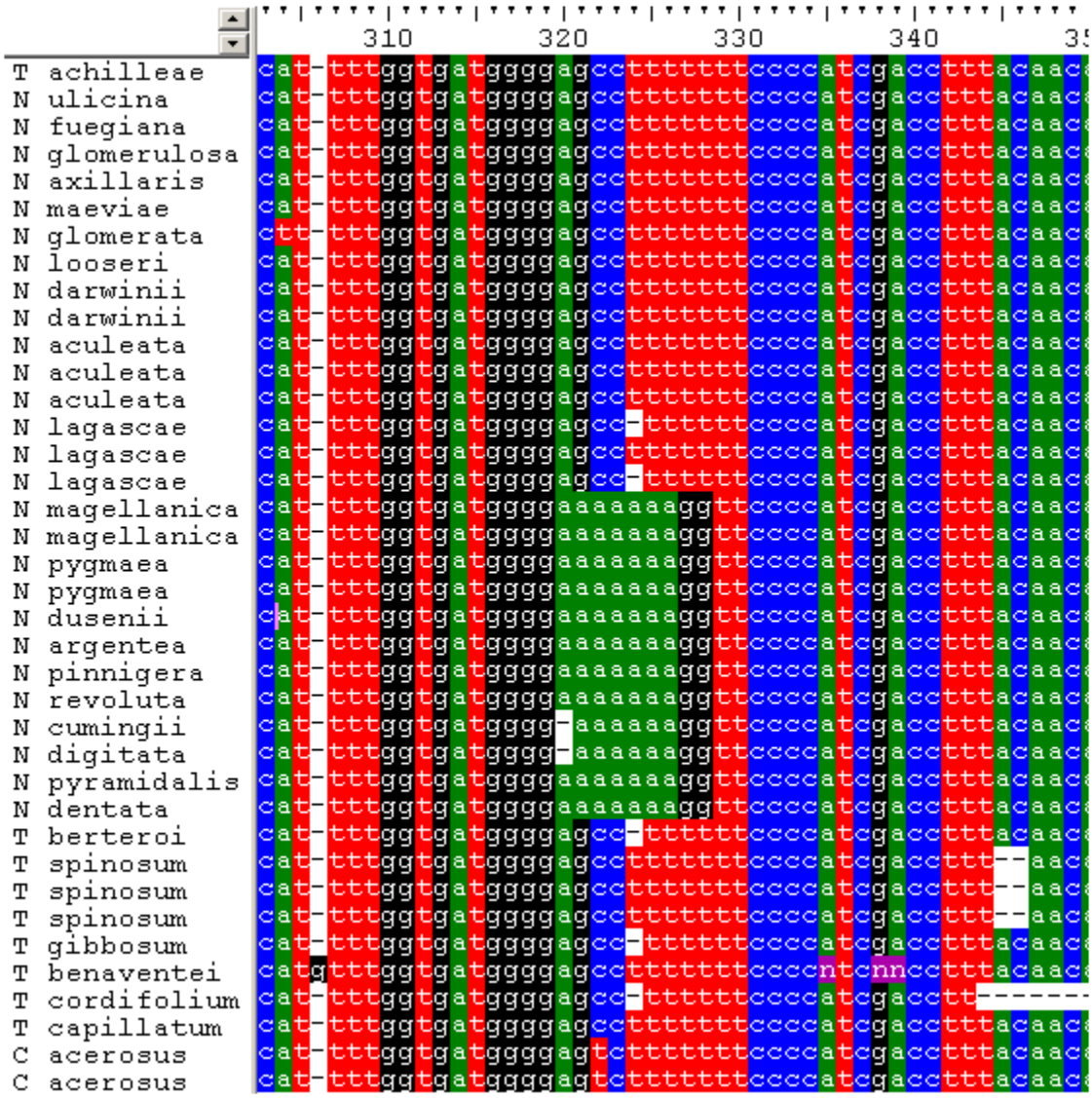

310
320
330
340
T achilleae
N ulicina
N fuegiana
N glomerulosa
N axillaris
N maeviae
N glomerata
N looseri
N darwinii
N darwinii
N aculeata
N aculeata
N aculeata
N lagascae
N lagascae
N lagascae
N magellanica
N magellanica
N pygmaea
N pygmaea
N dusenii
N argentea
N pinnigera
N revoluta
N cumingii
N digitata
N pyramidalis
N dentata
T berteroi
T spinosum
T spinosum
T spinosum
T gibbosum
T benaventei
T cordifolium
T capillatum
C acerosus
C acerosus

Figure 2

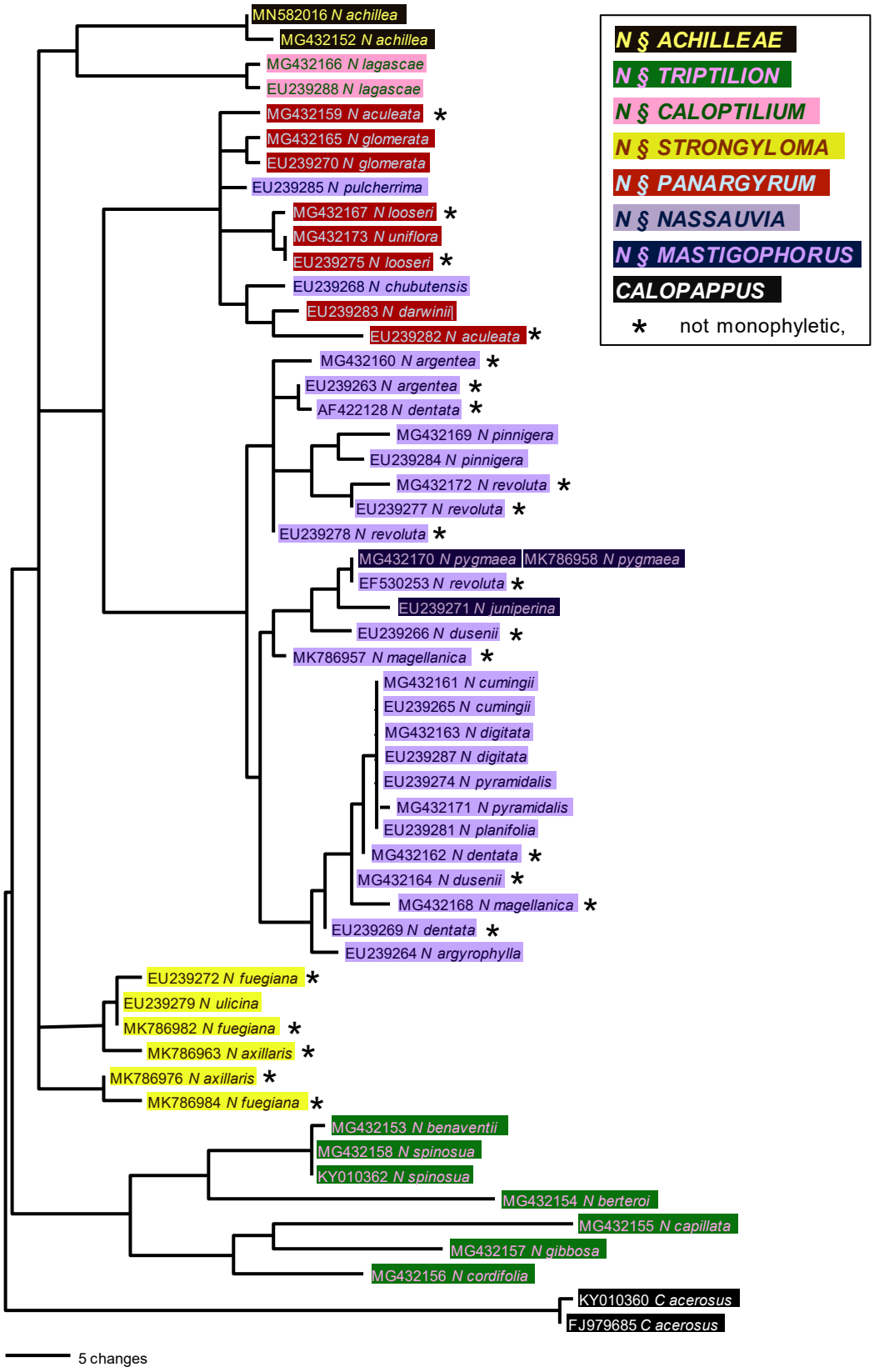

MN582016 N achillea
MG432152 N achillea
MG432166 N lagascae
EU239288 N lagascae
MG432159 N aculeata *
MG432165 N glomerata
EU239270 N glomerata
EU239285 N pulcherrima
MG432167 N looseri *
MG432173 N uniflora
EU239275 N looseri *
EU239268 N chubutensis
EU239283 N darwinii
EU239282 N aculeata *
MG432160 N argentea *
EU239263 N argentea *
AF422128 N dentata *
MG432169 N pinnigera
EU239284 N pinnigera
MG432172 N revoluta *
EU239277 N revoluta *
EU239278 N revoluta *
MG432170 N pygmaea
MK786958 N pygmaea
EF530253 N revoluta *
EU239271 N juniperina
EU239266 N dusenii *
MK786957 N magellanica *
MG432161 N cumingii
EU239265 N cumingii
MG432163 N digitata
EU239287 N digitata
EU239274 N pyramidalis
MG432171 N pyramidalis
EU239281 N planifolia
MG432162 N dentata *
MG432164 N dusenii *
MG432168 N magellanica *
EU239269 N dentata *
EU239264 N argyrophylla
EU239272 N fuegiana *
EU239279 N ulicina
MK786982 N fuegiana *
MK786963 N axillaris *
MK786976 N axillaris *
MK786984 N fuegiana *
MG432153 N benaventii
MG432158 N spinosua
KY010362 N spinosua
MG432154 N berteroi
MG432155 N capillata
MG432157 N gibbosa
MG432156 N cordifolia
KY010360 C acerosus
FJ979685 C acerosus
5 changes
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
* not monophyletic,

Figure 3

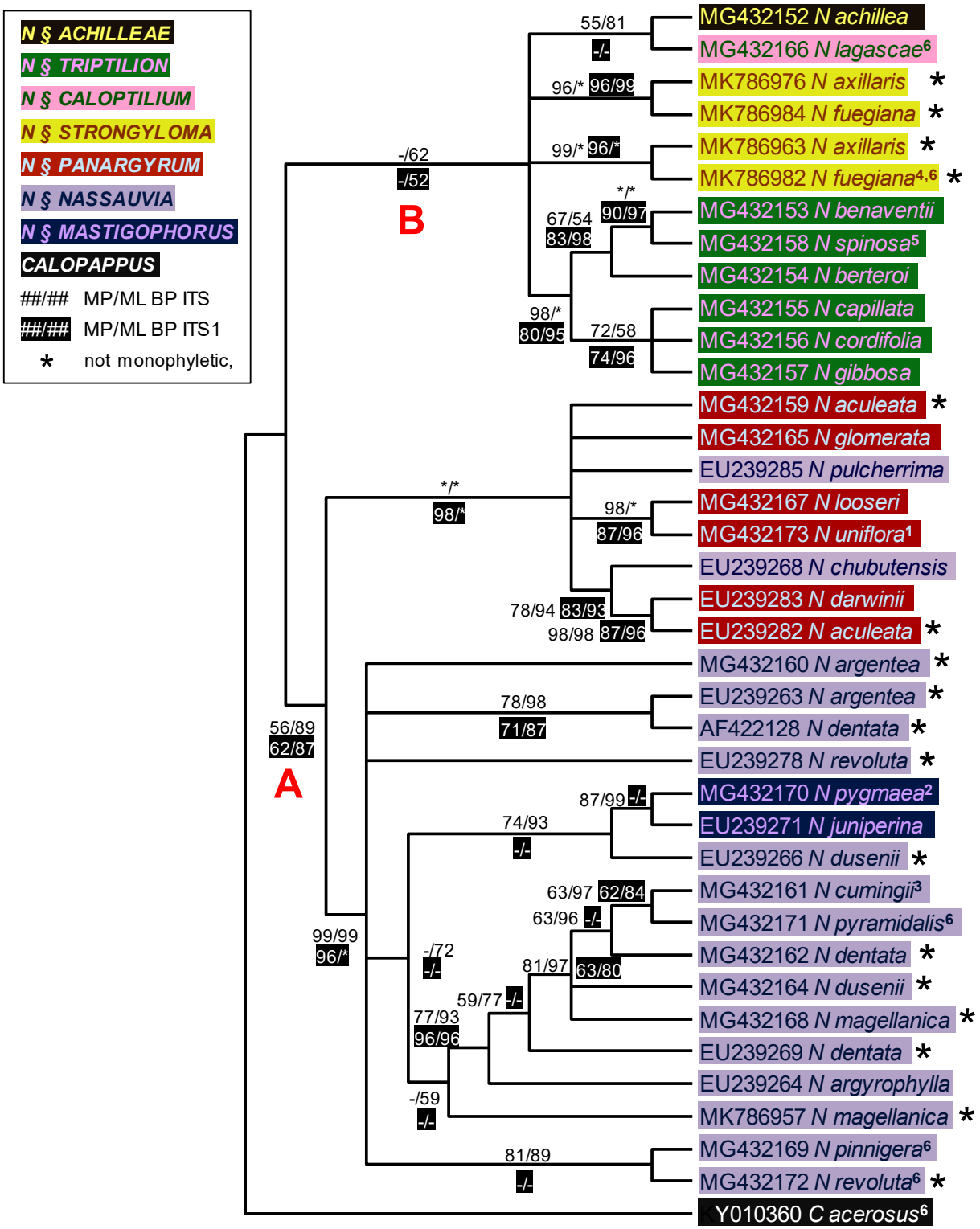

N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML BP ITS
##/## MP/ML BP ITS1
* not monophyletic,
MG432152 N achillea
MG432166 N lagascae6
MK786976 N axillaris *
MK786984 N fuegiana *
MK786963 N axillaris *
MK786982 N fuegiana4,6 *
MG432153 N benaventii
MG432158 N spinosa5
MG432154 N berteroi
MG432155 N capillata
MG432156 N cordifolia
MG432157 N gibbosa
MG432159 N aculeata *
MG432165 N glomerata
EU239285 N pulcherrima
MG432167 N looseri
MG432173 N uniflora1
EU239268 N chubutensis
EU239283 N darwinii
EU239282 N aculeata *
MG432160 N argentea *
EU239263 N argentea *
AF422128 N dentata *
EU239278 N revoluta *
MG432170 N pygmaea2
EU239271 N juniperina
EU239266 N dusenii *
MG432161 N cumingii3
MG432171 N pyramidalis6
MG432162 N dentata *
MG432164 N dusenii *
MG432168 N magellanica *
EU239269 N dentata *
EU239264 N argyrophylla
MK786957 N magellanica *
MG432169 N pinnigera6
MG432172 N revoluta6 *
Y010360 C acerosus6
55/81
-/-
96/* 96/99
99/* 96/*
-/62
-/52
B
/
90/97
67/54
83/98
98/*
80/95
72/58
74/96
/
98/*
98/*
87/96
78/94 83/93
98/98 87/96
78/98
71/87
56/89
62/87
A
87/99 -/-
74/93
-/-
63/97 62/84
63/96 -/-
99/99
96/*
-/72
-/-
81/97
63/80
59/77 -/-
77/93
96/96
-/59
-/-
81/89
-/-

Figure 4

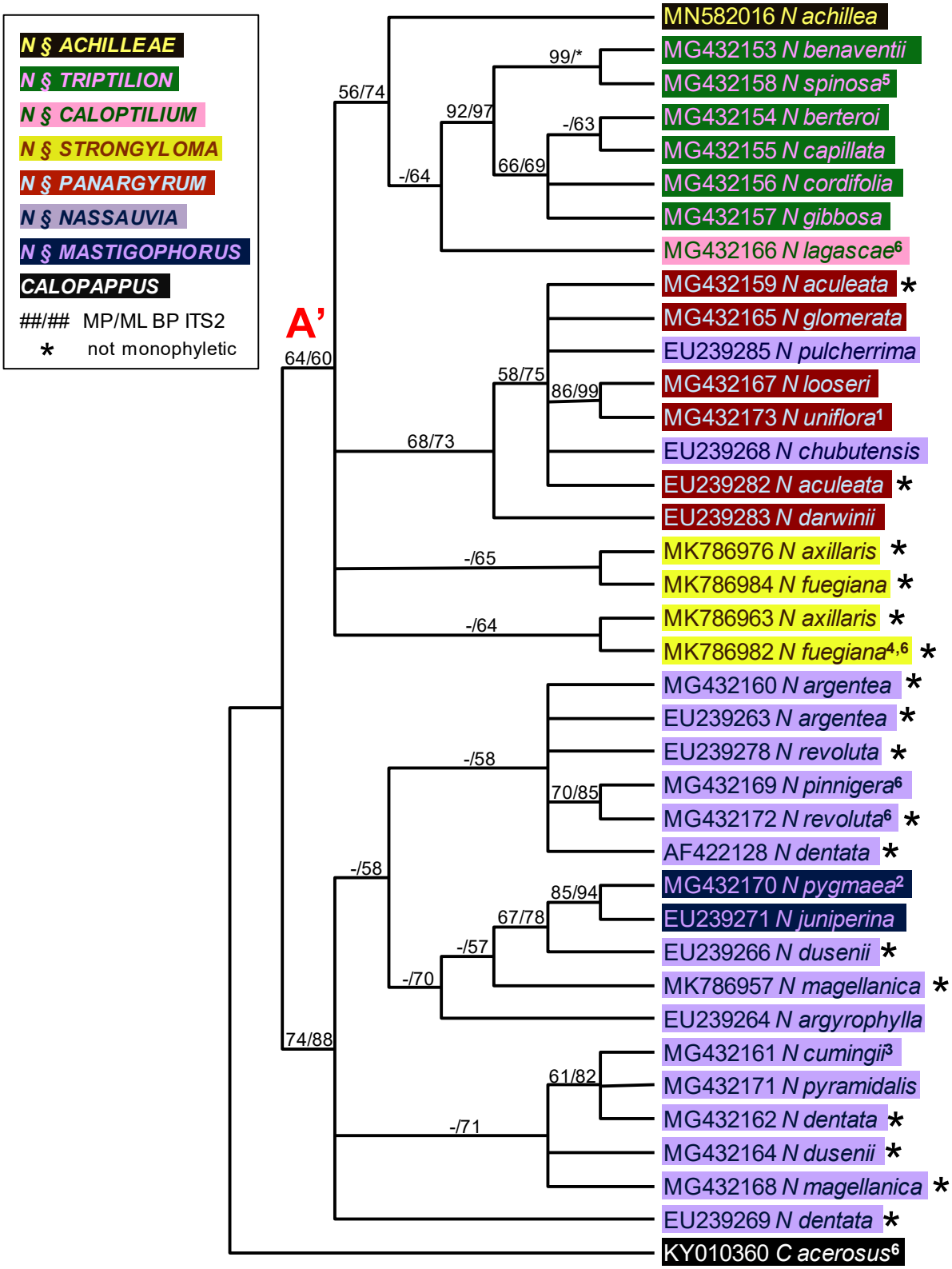

N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML BP ITS2
* not monophyletic
A'
MN582016 N achillea
MG432153 N benaventii
MG432158 N spinosa5
MG432154 N berteroi
MG432155 N capillata
MG432156 N cordifolia
MG432157 N gibbosa
MG432166 N lagascae6
MG432159 N aculeata *
MG432165 N glomerata
EU239285 N pulcherrima
MG432167 N looseri
MG432173 N uniflora1
EU239268 N chubutensis
EU239282 N aculeata *
EU239283 N darwinii
MK786976 N axillaris *
MK786984 N fuegiana *
MK786963 N axillaris *
MK786982 N fuegiana4,6 *
MG432160 N argentea *
EU239263 N argentea *
EU239278 N revoluta *
MG432169 N pinnigera6
MG432172 N revoluta6 *
AF422128 N dentata *
MG432170 N pygmaea2
EU239271 N juniperina
EU239266 N dusenii *
MK786957 N magellanica *
EU239264 N argyrophylla
MG432161 N cumingii3
MG432171 N pyramidalis
MG432162 N dentata *
MG432164 N dusenii *
MG432168 N magellanica *
EU239269 N dentata *
KY010360 C acerosus6
99/*
56/74
92/97
-/63
-/64
66/69
64/60
58/75
86/99
68/73
-/65
-/64
-/58
70/85
-/58
85/94
67/78
-/57
-/70
74/88
61/82
-/71

Figure 5

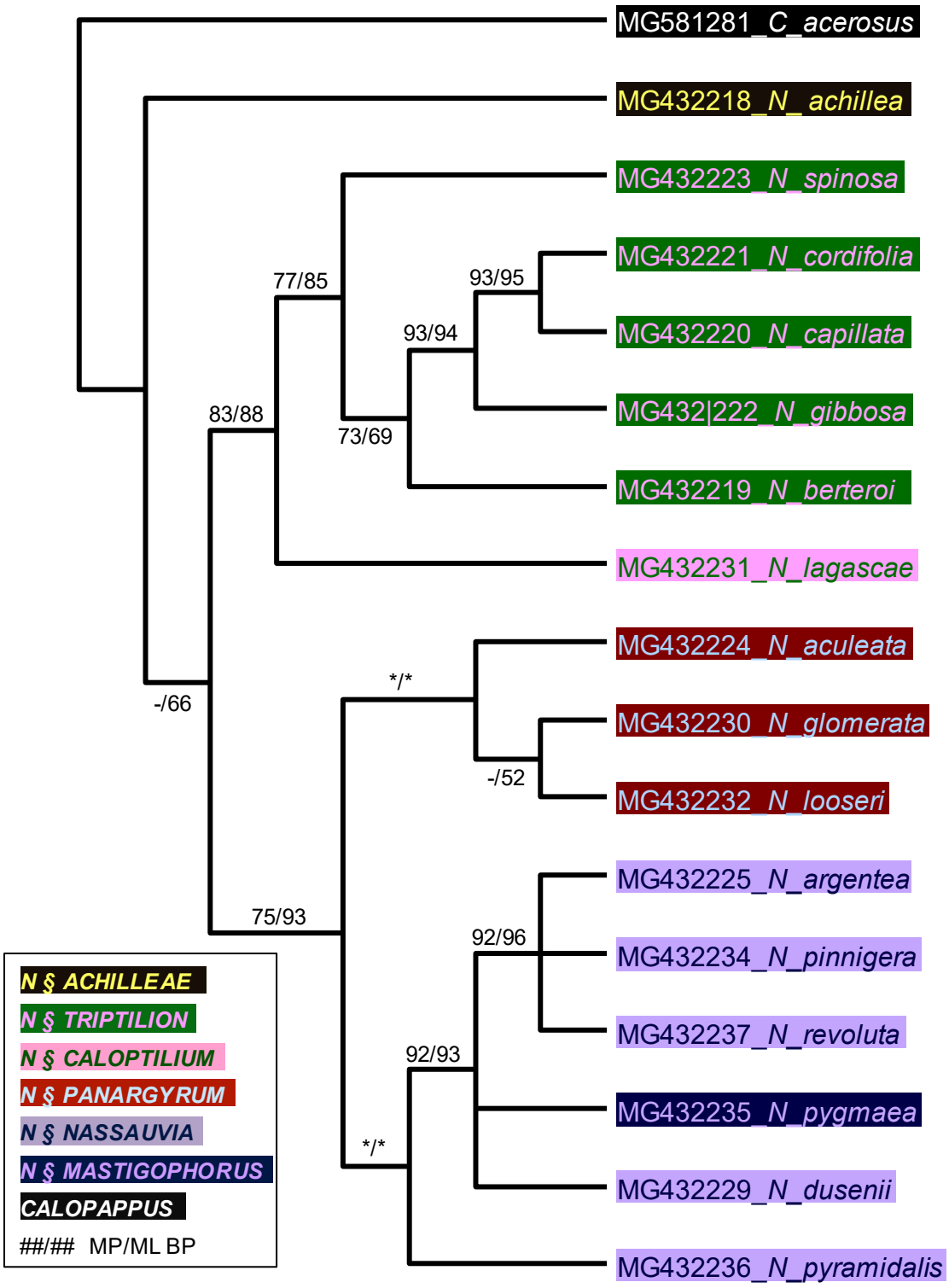

MG581281_C_acerosus
MG432218_N_achillea
MG432223_N_spinosa
MG432221_N_cordifolia
MG432220_N_capillata
MG432|222_N_gibbosa
MG432219_N_berteroi
MG432231_N_lagascae
MG432224_N_aculeata
MG432230_N_glomerata
MG432232_N_looseri
MG432225_N_argentea
MG432234_N_pinnigera
MG432237_N_revoluta
MG432235_N_pygmaea
MG432229_N_dusenii
MG432236_N_pyramidalis
77/85
93/95
93/94
83/88
73/69
-/66
/
-/52
75/93
92/96
92/93
/
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML BP

Figure 6

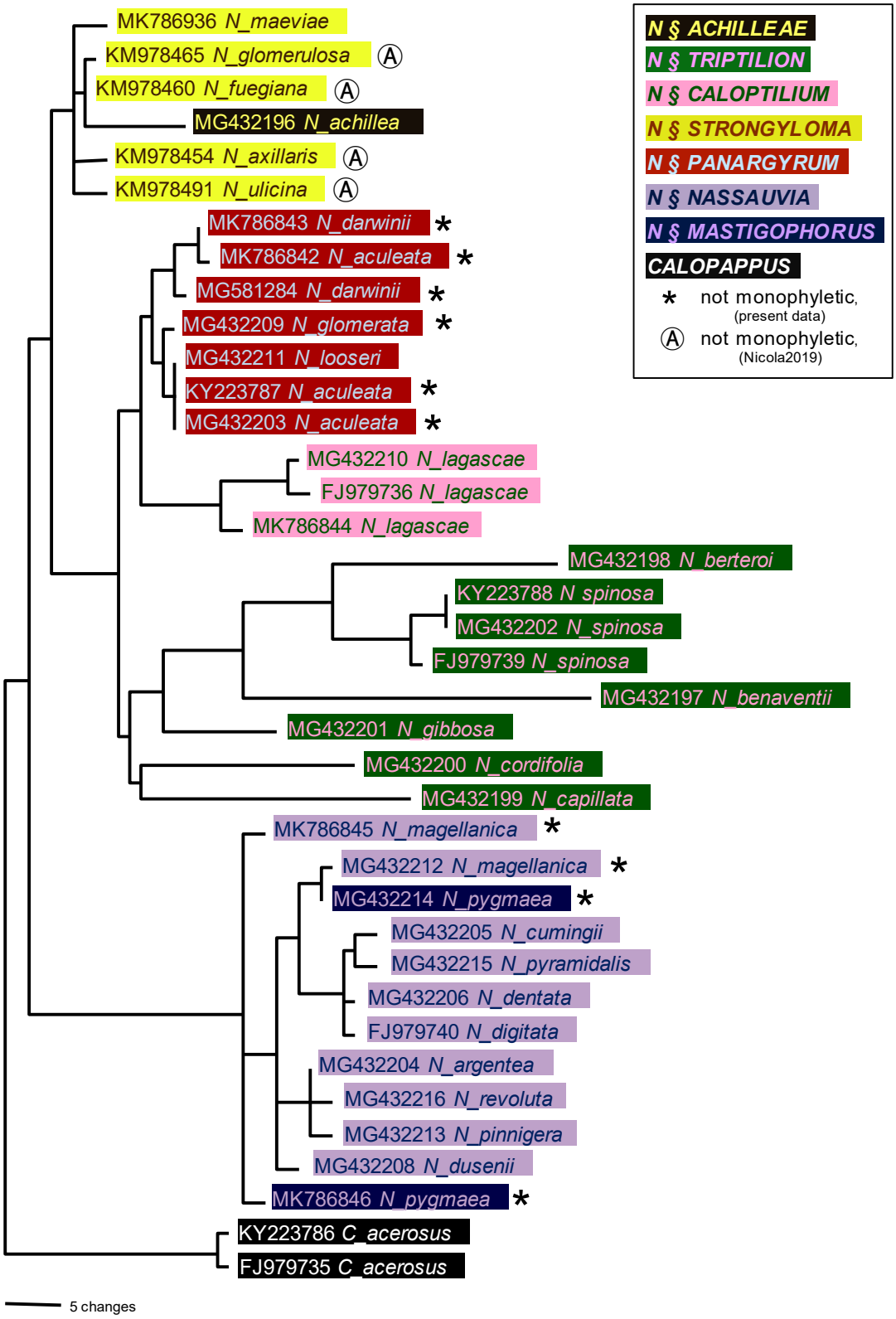

MK786936 N_maeviae
KM978465 N_glomerulosa Ⓐ
KM978460 N_fuegiana Ⓐ
MG432196 N_achillea
KM978454 N_axillaris Ⓐ
KM978491 N_ulicina Ⓐ
MK786843 N_darwinii *
MK786842 N_aculeata *
MG581284 N_darwinii *
MG432209 N_glomerata *
MG432211 N_looseri
KY223787 N_aculeata *
MG432203 N_aculeata *
MG432210 N_lagascae
FJ979736 N_lagascae
MK786844 N_lagascae
MG432198 N_berteroi
KY223788 N spinosa
MG432202 N_spinosa
FJ979739 N_spinosa
MG432197 N_benaventii
MG432201 N_gibbosa
MG432200 N_cordifolia
MG432199 N_capillata
MK786845 N_magellanica *
MG432212 N_magellanica *
MG432214 N_pygmaea *
MG432205 N_cumingii
MG432215 N_pyramidalis
MG432206 N_dentata
FJ979740 N_digitata
MG432204 N_argentea
MG432216 N_revoluta
MG432213 N_pinnigera
MG432208 N_dusenii
MK786846 N_pygmaea *
KY223786 C_acerosus
FJ979735 C_acerosus
5 changes
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
* not monophyletic, (present data)
Ⓐ not monophyletic, (Nicola2019)

Figure 7

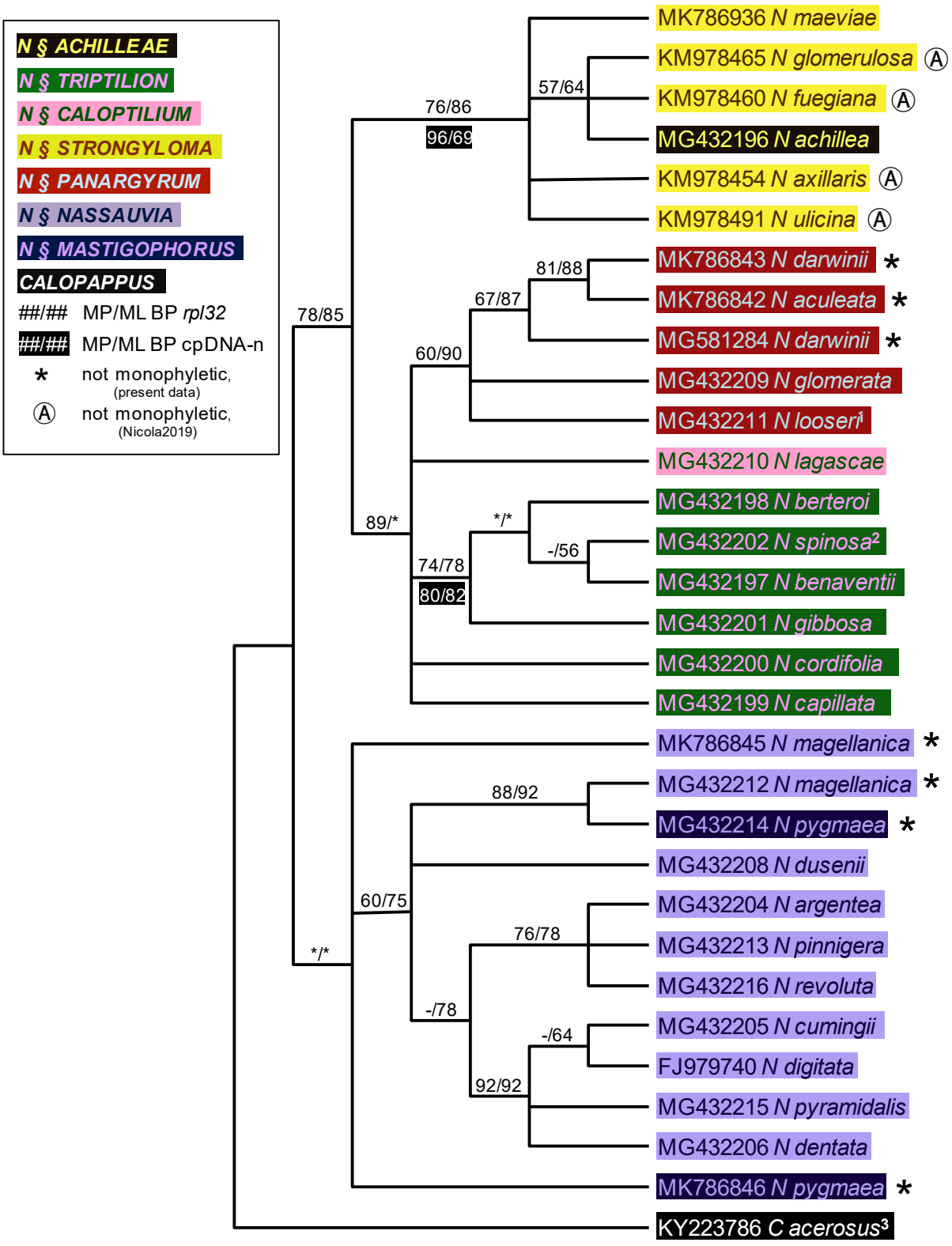

N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML BP rpl32
##/## MP/ML BP cpDNA-n
* not monophyletic, (present data)
Ⓐ not monophyletic, (Nicola2019)
MK786936 N maeviae
KM978465 N glomerulosa Ⓐ
KM978460 N fuegiana Ⓐ
MG432196 N achillea
KM978454 N axillaris Ⓐ
KM978491 N ulicina Ⓐ
MK786843 N darwinii *
MK786842 N aculeata *
MG581284 N darwinii *
MG432209 N glomerata
MG432211 N looseri[1]
MG432210 N lagascae
MG432198 N berteroi
MG432202 N spinosa[2]
MG432197 N benaventii
MG432201 N gibbosa
MG432200 N cordifolia
MG432199 N capillata
MK786845 N magellanica *
MG432212 N magellanica *
MG432214 N pygmaea *
MG432208 N dusenii
MG432204 N argentea
MG432213 N pinnigera
MG432216 N revoluta
MG432205 N cumingii
FJ979740 N digitata
MG432215 N pyramidalis
MG432206 N dentata
MK786846 N pygmaea *
KY223786 C acerosus[3]
57/64
76/86
96/69
81/88
67/87
78/85
60/90
89/*
/
-/56
74/78
80/82
88/92
60/75
/
76/78
-/78
-/64
92/92

Figure 8

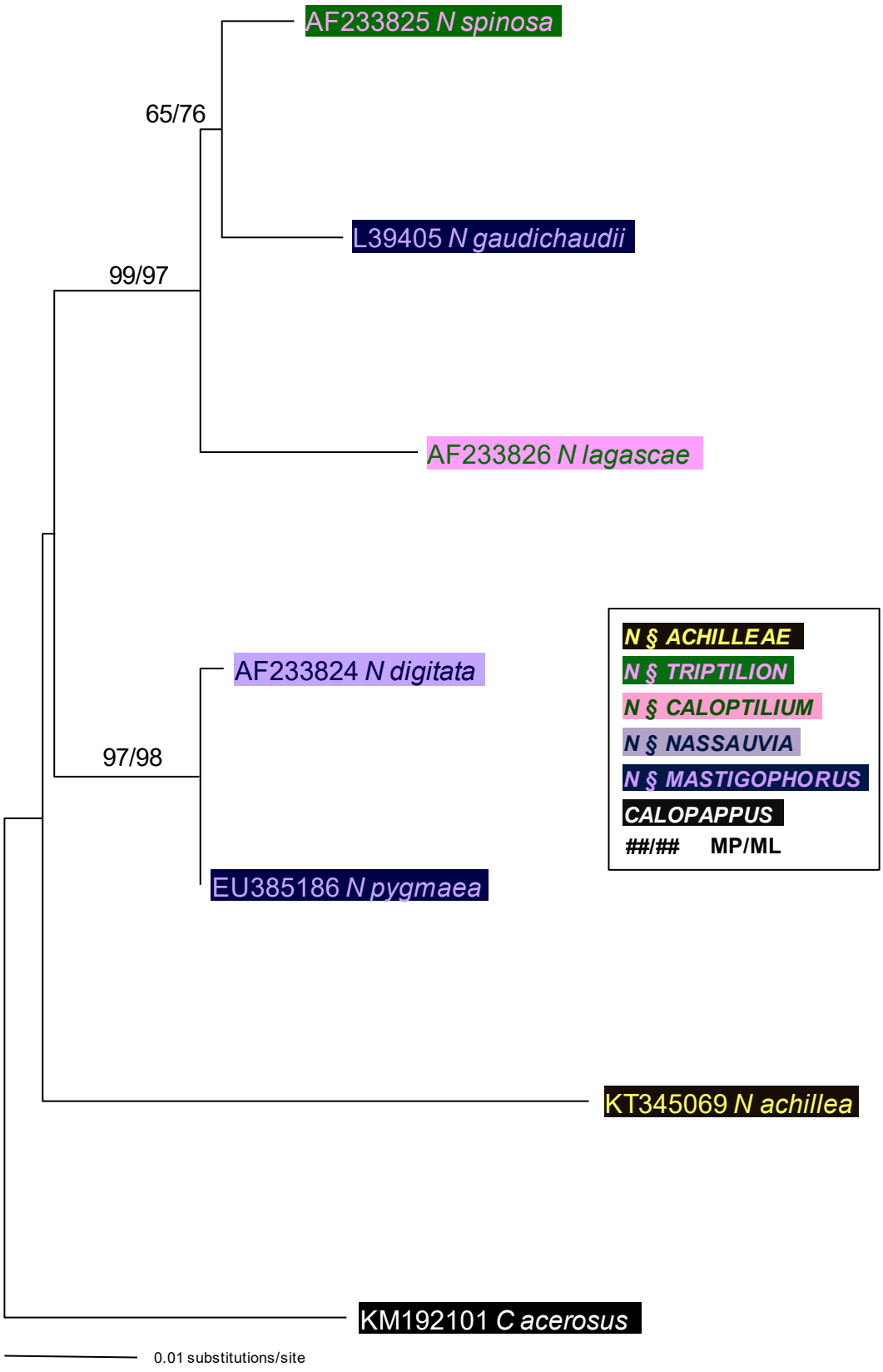

AF233825 N spinosa
65/76
L39405 N gaudichaudii
99/97
AF233826 N lagascae
AF233824 N digitata
97/98
EU385186 N pygmaea
KT345069 N achillea
KM192101 C acerosus
0.01 substitutions/site
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML

Figure 9

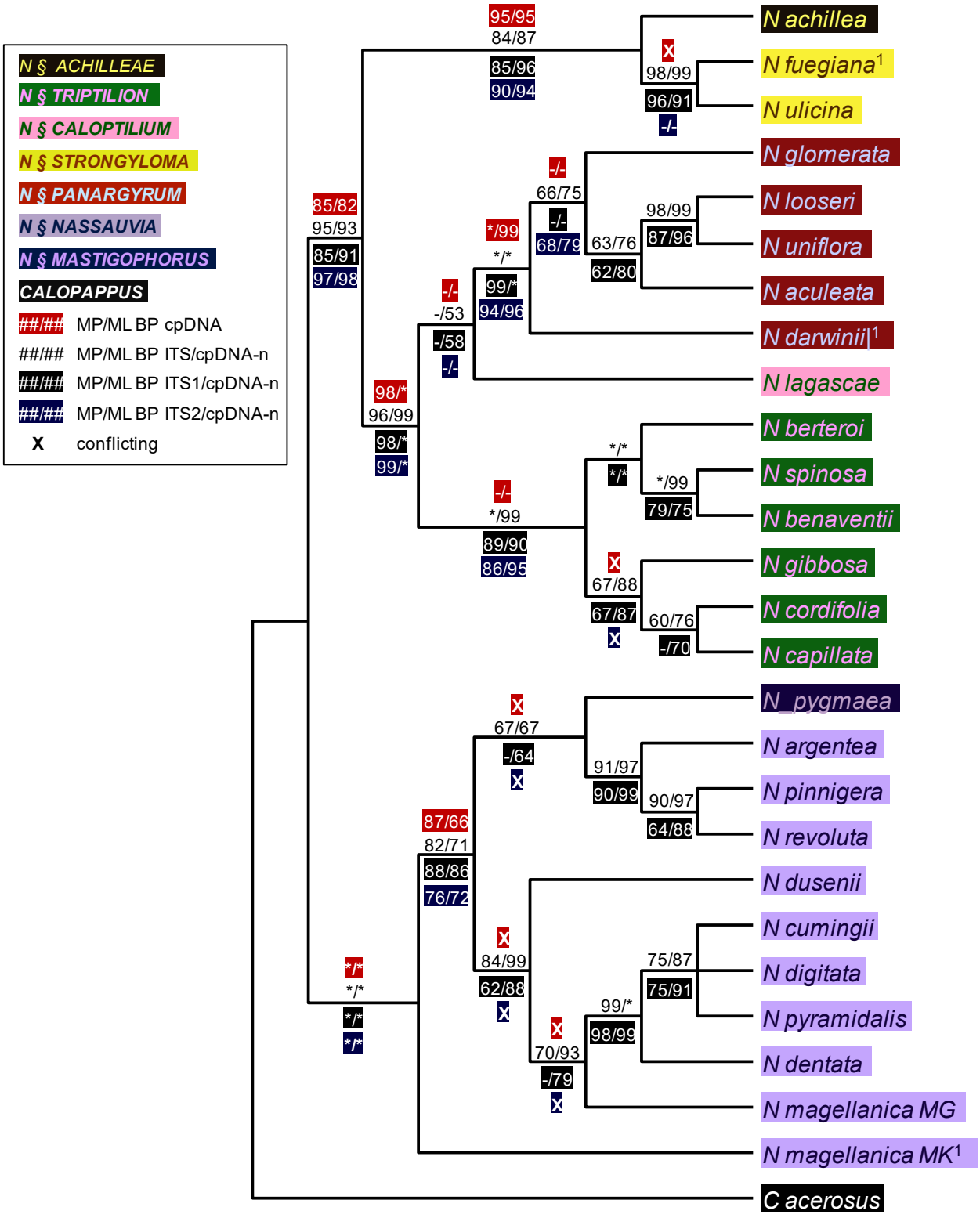

N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
##/## MP/ML BP cpDNA
##/## MP/ML BP ITS/cpDNA-n
##/## MP/ML BP ITS1/cpDNA-n
##/## MP/ML BP ITS2/cpDNA-n
X conflicting
N achillea
N fuegiana[1]
N ulicina
N glomerata
N looseri
N uniflora
N aculeata
N darwinii[1]
N lagascae
N berteroi
N spinosa
N benaventii
N gibbosa
N cordifolia
N capillata
N_pygmaea
N argentea
N pinnigera
N revoluta
N dusenii
N cumingii
N digitata
N pyramidalis
N dentata
N magellanica MG
N magellanica MK[1]
C acerosus

Figure 10A

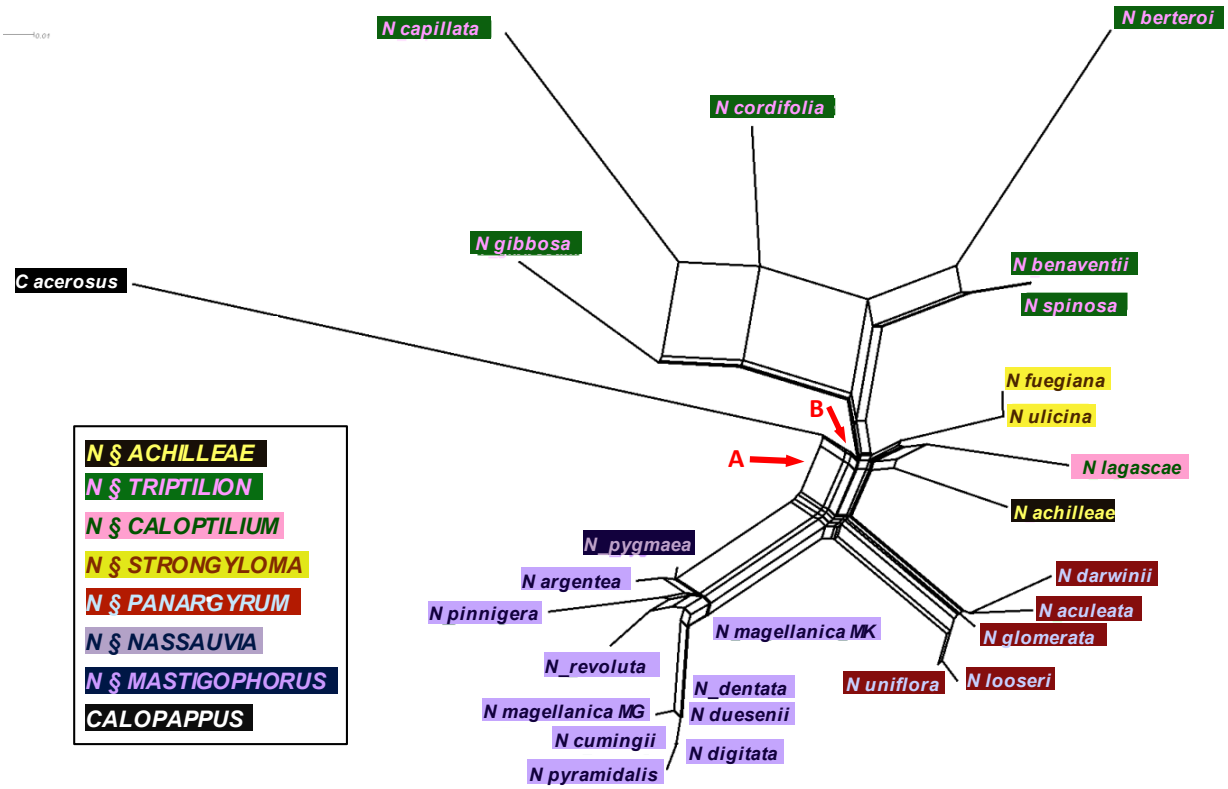

N capillata
N berteroi
N cordifolia
N gibbosa
C acerosus
N benaventii
N spinosa
N fuegiana
N ulicina
B
A
N lagascae
N achilleae
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
N_pygmaea
N argentea
N pinnigera
N darwinii
N aculeata
N glomerata
N magellanica MK
N_revoluta
N uniflora
N looseri
N_dentata
N magellanica MG
N duesenii
N cumingii
N digitata
N pyramidalis

Figure 10B

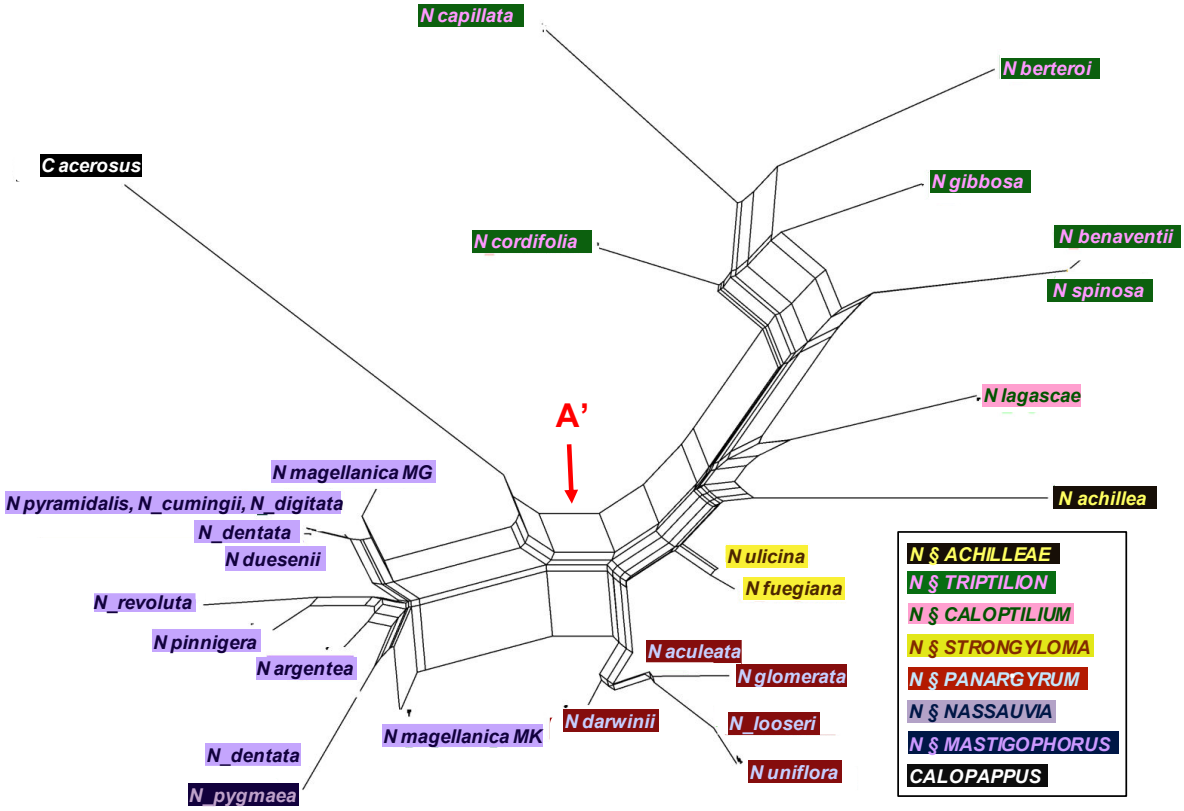

N capillata
N berteroi
C acerosus
N gibbosa
N cordifolia
N benaventii
N spinosa
A'
N lagascae
N magellanica MG
N achillea
N pyramidalis, N_cumingii, N_digitata
N_dentata
N duesenii
N ulicina
N fuegiana
N_revoluta
N pinnigera
N argentea
N aculeata
N glomerata
N darwinii
N_looseri
N magellanica MK
N_dentata
N uniflora
N_pygmaea
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS

Figure 10C

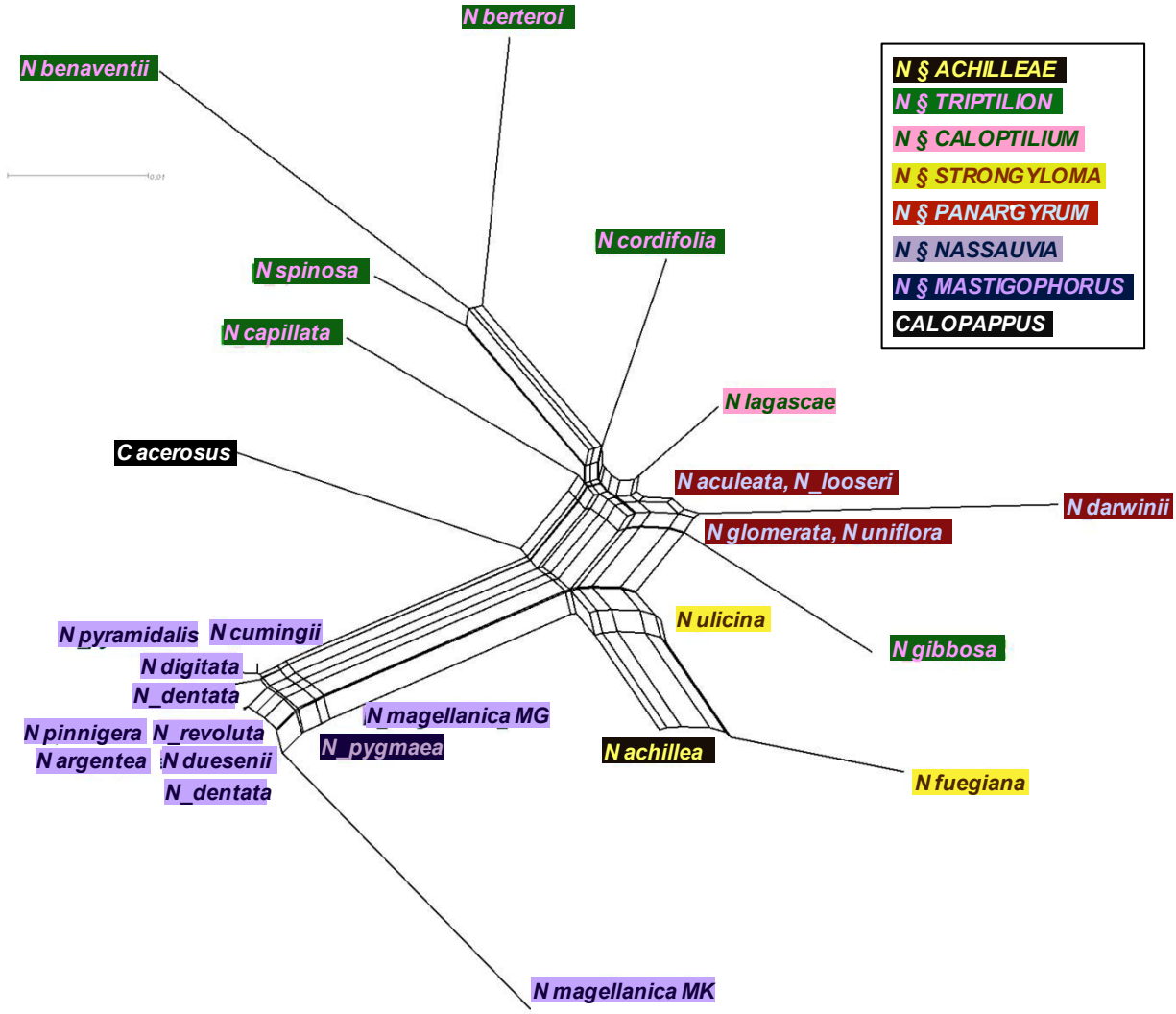

N berteroi
N benaventii
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
N cordifolia
N spinosa
N capillata
N lagascae
C acerosus
N aculeata, N_looseri
N darwinii
N glomerata, N uniflora
N ulicina
N gibbosa
N pyramidalis
N cumingii
N digitata
N_dentata
N magellanica MG
N pinnigera
N_revoluta
N_pygmaea
N argentea
N duesenii
N achillea
N fuegiana
N_dentata
N magellanica MK

Figure 10D

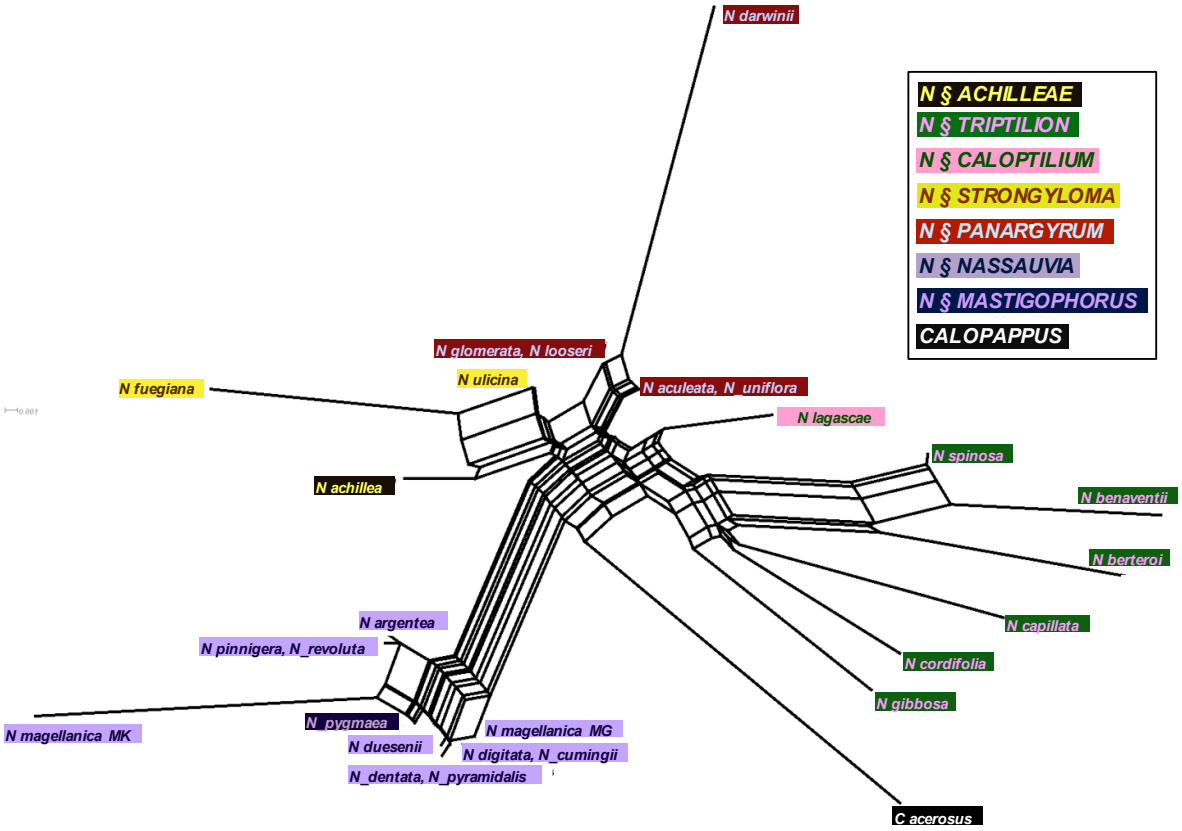

N darwinii
N § ACHILLEAE
N § TRIPTILION
N § CALOPTILIUM
N § STRONGYLOMA
N § PANARGYRUM
N § NASSAUVIA
N § MASTIGOPHORUS
CALOPAPPUS
N glomerata, N looseri
N ulicina
N fuegiana
N aculeata, N uniflora
N lagascae
N spinosa
N achillea
N benaventii
N berteroi
N capillata
N argentea
N pinnigera, N revoluta
N cordifolia
N gibbosa
N pygmaea
N magellanica MK
N magellanica MG
N duesenii
N digitata, N cumingii
N dentata, N pyramidalis
C acerosus

Figure 11

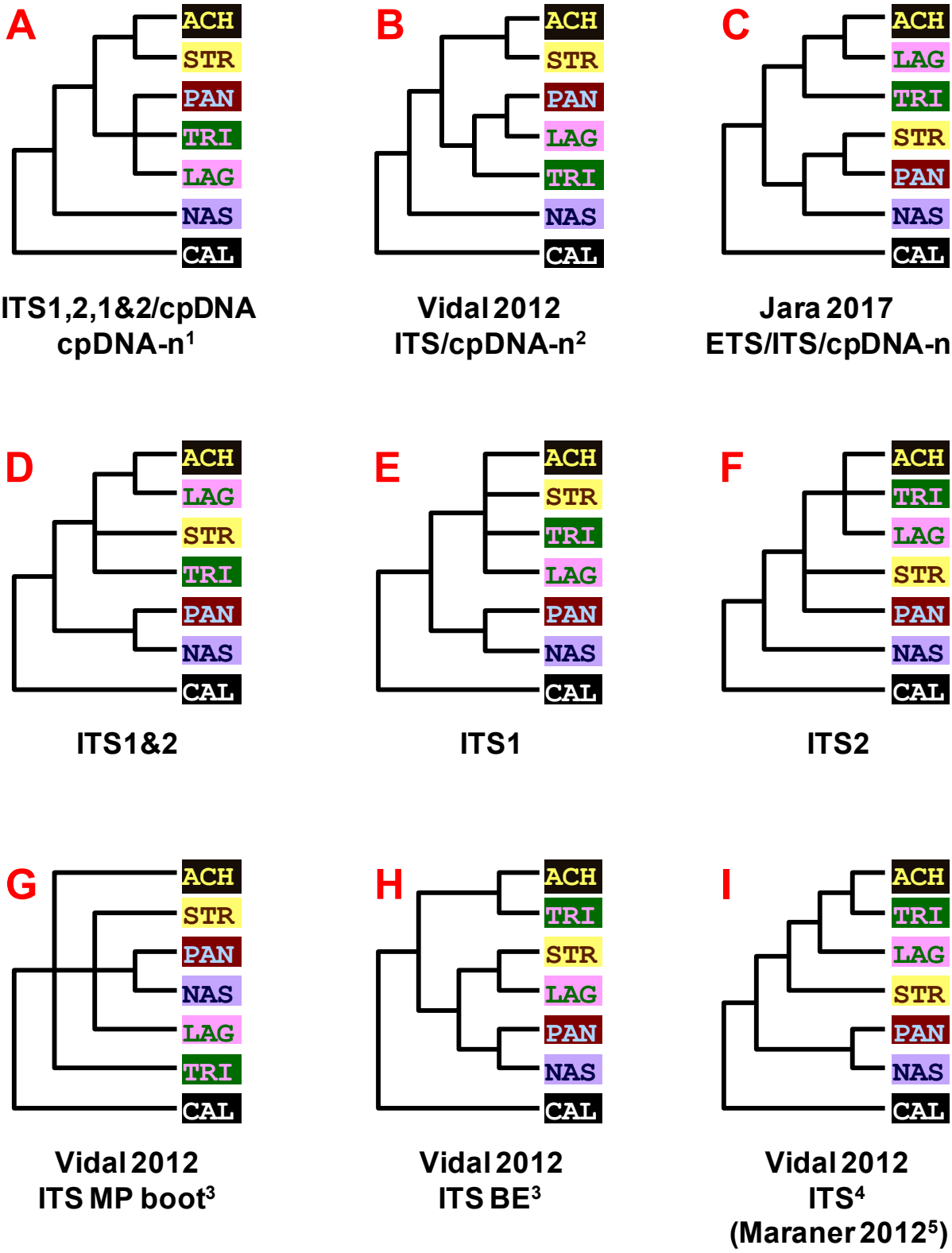

A
ACH
STR
PAN
TRI
LAG
NAS
CAL
ITS1,2,1&2/cpDNA
cpDNA-n[1]
B
ACH
STR
PAN
LAG
TRI
NAS
CAL
Vidal 2012
ITS/cpDNA-n[2]
C
ACH
LAG
TRI
STR
PAN
NAS
CAL
Jara 2017
ETS/ITS/cpDNA-n
D
ACH
LAG
STR
TRI
PAN
NAS
CAL
ITS1&2
E
ACH
STR
TRI
LAG
PAN
NAS
CAL
ITS1
F
ACH
TRI
LAG
STR
PAN
NAS
CAL
ITS2
G
ACH
STR
PAN
NAS
LAG
TRI
CAL
Vidal 2012
ITS MP boot[3]
H
ACH
TRI
STR
LAG
PAN
NAS
CAL
Vidal 2012
ITS BE[3]
I
ACH
TRI
LAG
STR
PAN
NAS
CAL
Vidal 2012
ITS[4]
(Maraner 2012[5])

Figure 12

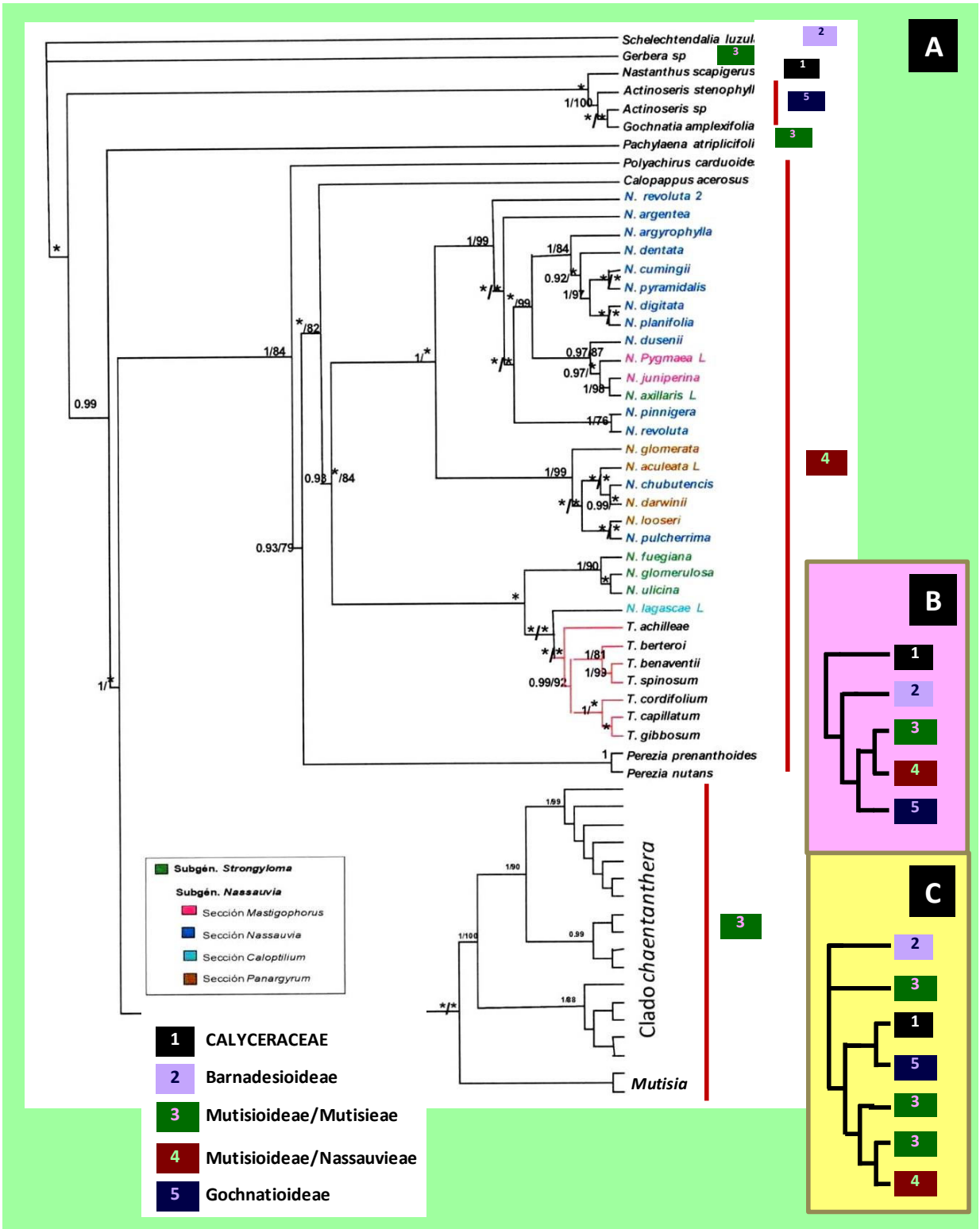
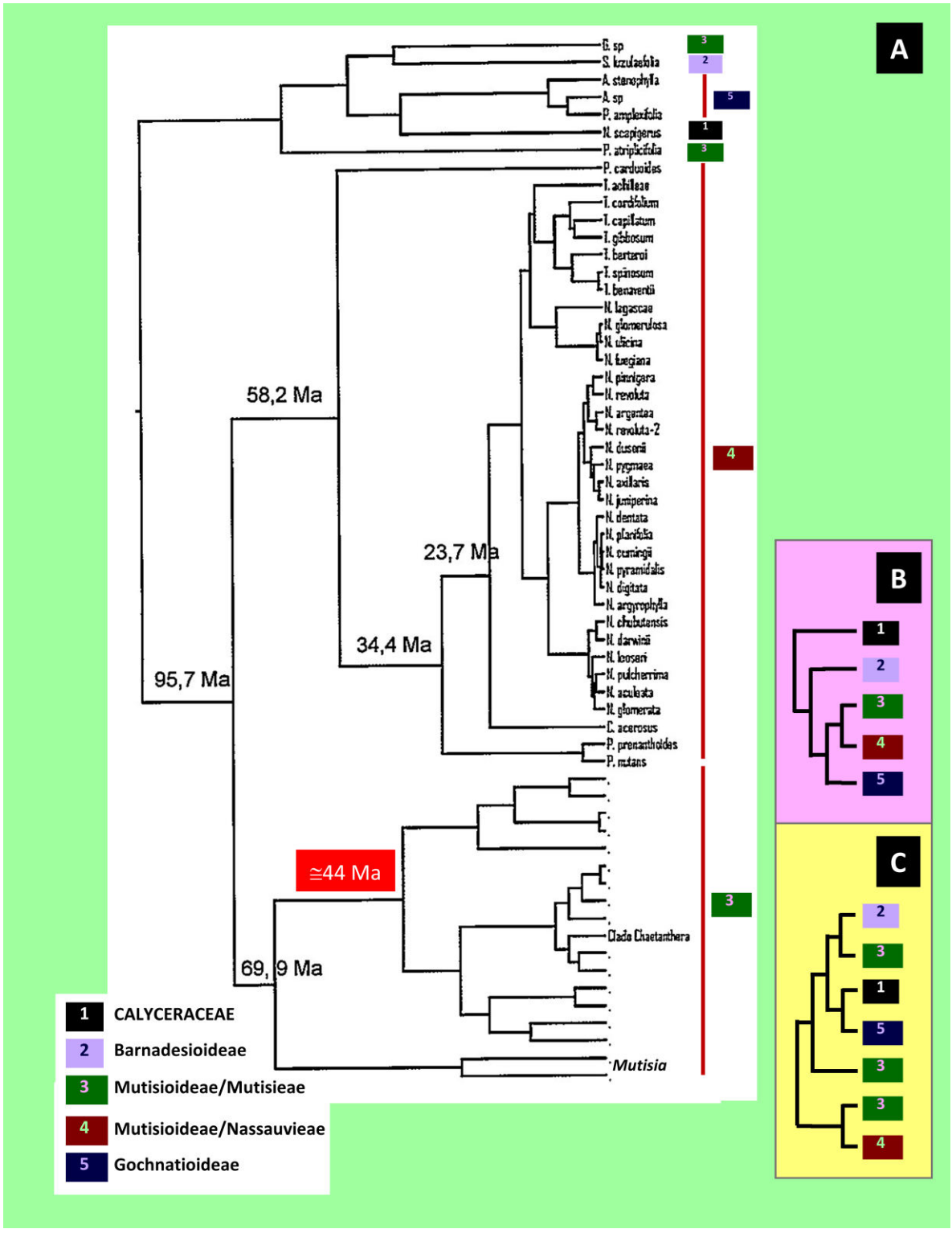

A
B
C
Schelechtendalia luzul
Gerbera sp
Nastanthus scapigerus
Actinoseris stenophyll
Actinoseris sp
Gochnatia amplexifolia
Pachylaena atriplicifoli
Polyachirus carduoide
Calopappus acerosus
N. revoluta 2
N. argentea
N. argyrophylla
N. dentata
N. cumingii
N. pyramidalis
N. digitata
N. planifolia
N. dusenii
N. Pygmaea L
N. juniperina
N. axillaris L
N. pinnigera
N. revoluta
N. glomerata
N. aculeata L
N. chubutencis
N. darwinii
N. looseri
N. pulcherrima
N. fuegiana
N. glomerulosa
N. ulicina
N. lagascae L
T. achilleae
T. berteroi
T. benaventii
T. spinosum
T. cordifolium
T. capillatum
T. gibbosum
Perezia prenanthoides
Perezia nutans
Clado chaentanthera
Mutisia
Subgén. Strongyloma
Subgén. Nassauvia
Sección Mastigophorus
Sección Nassauvia
Sección Caloptilium
Sección Panargyrum
1 CALYCERACEAE
2 Barnadesioideae
3 Mutisioideae/Mutisieae
4 Mutisioideae/Nassauvieae
5 Gochnatioideae

Figure 13


A
G. sp
S. kuzulaefolia
A. stenophylla
A. sp
P. amplexifolia
N. scapigerus
P. atriplicifolia
P. cardunculus
I. achilleae
I. cordifolium
I. capillatum
I. gibbosum
I. berterii
I. spinosum
I. benaventii
N. lagascae
N. glomerulosa
N. ulicina
N. fuegiana
N. pinnigera
N. revoluta
N. argentea
N. revoluta-2
N. dusenii
N. pygmaea
N. axillaris
N. juniperina
N. dentata
N. planifolia
N. cruzingii
N. pyramidalis
N. digitata
N. argyrophylla
N. chubutensis
N. darwinii
N. loosen
N. pulcherrima
N. aculeata
N. glomerata
C. acerosus
P. prenanthoides
P. mutans
Clade Chaetanthera
Mutisia
58,2 Ma
23,7 Ma
34,4 Ma
95,7 Ma
≅44 Ma
69,9 Ma
1 CALYCERACEAE
2 Barnadesioideae
3 Mutisioideae/Mutisieae
4 Mutisioideae/Nassauvieae
5 Gochnatioideae
B
C